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ACTIVE ANAPHYLAXIS IN THE CHICK EMBRYO

PRELIMINARY REPORT

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SHERWOOD[1] has shown that the embryonic 4-day-old chick heart can be passively sensitized by chick antiserum and that the anaphylactic response consists of a marked slowing of the whole heart with a decrease in amplitude and increase in diastole. Moreover, desensitization occurred as proved by no response upon further addition of the antigen. However, he obtained only about 15 per cent positive results.

Witebsky and Szepsenwol[2, 3] and later Baumann and Witebsky[4, 5] showed that from its earliest development the chick embryo and its vascular network contain Forssman's antigen and that the addition of normal human serum, rabbit serum, or Forssman's antisera causes a peculiar "vascular phenomenon" in which "the vascular network shrinks, the embryo turns around and sinks into the yolk," and the heart finally dies.

Later Witebsky and Neter[6, 7] used the chick embryo to demonstrate the mechanism of this primary serum toxicity and the influence of drugs on the chick embryo.

The practical problem of testing and hyposensitizing allergic patients has been considerably simplified by the knowledge of group or cross relationship of various antigens.

Gross anaphylaxis methods, the Dale test, and cross testing after exhaustion of passively sensitized sites offer certain handicaps. Except for pollens, a few of the mold species, and components of house dust, little has been done to systematically determine the immunologic relationship of the atmospheric allergens.

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A year ago the author commenced studies in an attempt to actively sensitize 3- to 4-day-old chick embryos with saline and isotonic dextrose protein extracts as well as with alum precipitates of various antigens and produced anaphylaxis with subsequent doses of the antigens two weeks later and after hatching.

TECHNIQUE

It is imperative that healthy chick embryos be obtained through strict standards of incubation and a stock selected from hens known to be free from disease and with a record for producing healthy fertile eggs rather than one for egg laying capacity. The eggs, preferably from Leghorns, were "candled" for fertility and placed in a small commercial electric chick incubator kept level at an even room temperature in a well ventilated room. They were turned four or five times daily after the second day of incubation by tilting from side to side at an angle of 30° to 40°, and the large end was always placed up. The eggs were incubated for three to eight days at a temperature of 100° to 101° F. and a humidity of 50 per cent. A hole in the end opposite the air sac was then made with a small sterile dental drill after the egg had been wiped with alcohol.

Some of the eggs were sensitized with 0.2 to 0.4 c.c. of 1 per cent alum precipitates, and others with saline or isotonic dextrose extracts of the various antigens by slowly injecting into the albumen just inside the shell. Control experiments were carried out by inoculating eggs the same amounts of physiologic solution of sodium chloride or isotonic dextrose when the latter was used as an extracting fluid. Tests were also made to determine the toxicity of Coca's solution, glycerine extracts, and isotonic dextrose solutions by injecting quantities as high as 0.75 c.c. These did not prevent hatching of apparently normal chicks. The site of the injection was covered with flamed Scotch tape. When the embryos were 14 to 18 days old, and also after hatched, various methods were tried to produce anaphylactic shock with saline extracts of various antigens.

METHODS OF PRODUCING ANAPHYLAXIS

Various methods were tried to produce anaphylaxis in the embryos after they were 14 to 18 days old. When the embryo can be clearly observed to be living when "candled," the "shock" dose of 0.3 to 0.5 c.c. can be directly injected. (These doses were found to be nontoxic in nonsensitized embryos.) With certain antigens the embryo will be observed to become more active and then die within five to ten minutes as confirmed by autopsy.

A second method was to inject in this manner, wait five minutes, break open the egg and expose the embryo heart and observe its movements in situ. In a third the embryo was removed and the yolk sac

injected. A fourth method was to expose the heart and drop the antigen directly onto the heart or inject it intravenously. A fifth method was to remove the chick quickly, open the chest, excise the heart and place it in 20 c.c. of modified Tyrode's solution or Locke's solution at 100° to 101° F. temperature and a pH of about 7.2. It was necessary to wait one or two minutes until the rate of pulsation became established and regular before the addition of the antigen. In the excising of the heart, the length of the vessels attached to it seemed to be a determining factor. If the vessels were left too long, the walls collapsed and the pulsation seemed more labored. When the cut was quite close to the heart, leaving the merest stumps, the pulsation was of a better tone and the time for regulation greatly lessened.

ANTIGENS USED

Extracts of various mill dusts (wheat, oat, corn, rye, dirty feed mill dusts), various grain smuts, cottonseed, horse dander, horse serum, egg albumen, cow's milk, lactalbumin, wheat, tetanus antitoxin, diphtheria antitoxin, and streptococcus serum were used. In an effort to determine the cross relationship of the various grains and their corresponding smuts by means of the Dale test in another study, parallel experiments were done with the chick embryos.

RESULTS

Although there were definite anaphylactic responses in about one-third of the embryo hearts, the results so far have been disappointing since they were not uniform.

The anaphylactic response was a marked slowing of the heart and cardiac standstill in diastole.* Desensitization occurred as proved by no response upon the addition of more antigen. The response also developed in the majority of experiments under five minutes and as early as forty-five seconds with horse serum. The longest response was ten minutes. The control hearts lived under the same conditions from fifteen to thirty minutes.

There was no shock with concentrated histamine solutions. Upon the addition of 0.1 c.c. of a 1:1,000 dilution of acetylcholine or choline to the solution containing the excised heart there was a uniform, very rapid, and almost instant cessation of the heart in systole. This was noted by St. Went[8] on excised guinea pig hearts. He also showed that the choline group, when added to the sensitized isolated heart, would cause exactly the same alterations as does anaphylaxis except that the heart ceases in systole, especially when previously sensitized with a foreign protein.

F. G. Jones of the Biologic Division of the Lilly Research Laboratories in collaboration with the author in these experiments noted that

*Subsequent electrocardiographic studies of the isolated heart showed decreased amplitude and slowing in shock.

after injection of diphtheria and tetanus antitoxin the hearts in the diphtheria group were somewhat smaller than those in the control group, and in the tetanus group all of the hearts were very small. After the hearts were removed and tested with these antigens, it was found that the tetanus antitoxin gave the most severe reactions in both the diphtheria- and tetanus antitoxin-sensitized embryos. In order to get comparable values when the eggs were sensitized with tetanus antitoxin, a number of the eggs were also injected with a physiologic salt solution to be sure that the mechanical manipulation did not produce any sensitivity. When these hearts were removed and placed in the bath, they were treated in various ways, some by the addition of tetanus antitoxin, some with normal horse serum, while others were untreated. None of these hearts showed any stimulation or reaction upon the addition of a sensitizing agent. While none of our control hearts, which had not been sensitized, gave any reaction on the addition of sensitizing agents, we have at times found hearts which would slow down and then accelerate. This type of reaction might be confused at times with an anaphylactic reaction, if it just so happens that we added sensitizing agent to the bath in which this heart was located. It is more or less common knowledge that serum reactions are usually more mild with diphtheria antitoxin in humans, whereas tetanus antitoxin gives stronger reactions. Since the streptococcus serums give the worst, the latter will be used to sensitize eggs in further studies.

It was also noted that the embryos receiving control injections of isotonic dextrose solutions were larger and more vigorous.

Further nutritional studies suggested by these observations are being made.

CONCLUSIONS

1. Active sensitization of 3- to 8-day-old chick embryos apparently was accomplished with alum precipitates, and saline and isotonic dextrose extracts of proteins. Alum precipitates seemed to increase the sensitiveness of the embryos.

2. About one-third of the isolated chick embryo hearts so sensitized manifested anaphylactic responses fourteen to eighteen days after the sensitizing dose with a marked slowing of the heart, decreased amplitude, and cardiac standstill in diastole.

3. The experiments done so far have been disappointing and impractical when compared with other methods of anaphylaxis, especially the Dale test, in determining the immunologic relationship of antigens.

4. The studies have rewarded us with an increasing appreciation of the possibilities of using the chick embryo for determining serum and antitoxin toxicities as well as the influence of certain agents, including the vitamins, on nutrition and growth.

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DISCUSSION

DR. HAROLD WAGNER.—I should like to say that Dr. Rothman of Knoxville, Tenn., has demonstrated some pathologic findings in the chick embryo under the influence of various bacterial growths. I should like to ask Dr. Wittich whether it occurred to him to use such methods.

DR. F. W. WITTICH.—I am very grateful for those valuable suggestions and hope to carry out further observations. I might say that we are doing some further studies now on chickens to confirm Dr. Ratner's inheritance studies on guinea pigs. We are sensitizing a full-grown cock, mating it with a normal hen, and attempting to shock the 72-hour ova; sensitizing the hen, mating it with a normal cock, and noting the effect of the shocking dose on their ova; and also sensitizing both the male and female and studying their sensitized ova. The results so far have been quite confirmatory of Dr. Ratner's work on the guinea pig.

THE USE OF SULFONAMIDE DRUGS IN BRONCHIAL ASTHMA

Preliminary Report of 23 Cases

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THE use of sulfonamide compounds has been of tremendous value in certain infectious diseases. It was considered possible that they would benefit patients in whom an infectious element, e.g., acute bronchitis, complicates the asthmatic condition.

Studies were already in progress when our interest in this subject was heightened by three recent reports. Weil and Climo[1] reported seven cases of status asthmaticus complicated by respiratory infection. All but one had fever and leucocytosis. In five cases in which the sputa showed streptococci excellent results were obtained from sulfanilamide. Sulfathiazole cleared up one patient in whom the sputum revealed pneumococci. In the seventh case sulfanilamide failed and sulfapyridine was used because of the presence of pneumococci in the sputum. The patient recovered within five days. The total dosages in each of these patients varied from 2.5 to 41 Gm. Weil and Climo noted also that the percentage of eosinophiles increased after the infectious element disappeared. They recommended sulfanilamide for streptococcic sputum, sulfapyridine for that containing pneumococci, and sulfathiazole for cases in which staphylococci predominate.

Keeney[2] stated that sulfanilamide is effected only in infections produced by the following organisms: Lancefield's group A, B, and C strains of the hemolytic streptococcus, the *salivarius* and *mitis* strains of the alpha hemolytic streptococcus, meningococcus, gonococcus, clostridium *welchii*, *B. coli*, and proteus vulgaris. He recommended sulfapyridine for pneumococci and sulfathiazole for staphylococci. He further advised that the drugs be pushed until the blood concentration of sulfanilamide is 10 mg. per cent or more, and that of sulfapyridine and sulfathiazole, 5 mg. or more. He also pointed out the advisability of doing blood counts every day and of stopping the drug at once if any toxicity should develop. Keeney stated that he has had some encouraging results in a very small group of well chosen patients, but he gave no other data on the subject.

Very recently Turnbull[3] reported on intranasal therapy with a 5 per cent sodium salt of sulfathiazole. He had excellent results in forty-five cases of chronic sinusitis. His two failures occurred in patients with hay fever.

From Cook County and Wesley Memorial Hospitals.
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TABLE I

BRONCHIAL ASTHMA TREATED BY SULFANILAMIDE

NAME	AGE (YEARS)	SEX	COMPLICATIONS	HOSPITALIZED	ORGANISMS IN SPUTUM	TEMPERATURE (° F.)	W.B.C.	EOSINOPHILES (%)	TOTAL DOSAGE OF DRUG (GM.)	RESULTS
S. B.	37	F	Acute bronchitis	Yes	Mixed	100	12,250	2	30	Excellent and chest cleared
M. G.	67	F	Chronic bronchitis	No		Normal	9,600	7	16	Improved
E. O.	38	F		No		Normal			16	Worse
G. G.	40	F		Yes	Mixed	Normal	7,200	5	18	No relief
A. H.	59	M	Prostatitis	Yes	Mixed	Normal	8,550	5	58	No relief
E. R.	57	M	Chronic bronchitis	No	Mixed	Normal	6,100	2	30	Slight relief
B. B.	41	M		Yes	Streptococci and pneumococci	99.4	14,950	11	19 (level 3.89)	No relief
A. C.	42	F		No	Mixed	99	10,000	4	17	Improved
R. K.	49	F	Tonsillitis	Yes	Pneumococci	98.6	8,650	11	48.5	No relief; nausea and vomiting
C. U.	39	F		Yes		98.6	12,600	7	27	No relief; nausea and vomiting
M. C.	53	F		Yes	Pneumococci	98.6			20	No relief
E. M.	47	F	Bronchopneumonia	Yes		100	10,750		37	Died of pneumonia

TABLE II

BRONCHIAL ASTHMA TREATED BY SULFATHIAZOLE

NAME	AGE (YEARS)	SEX	COMPLICATIONS	HOSPITALIZED	ORGANISMS IN SPUTUM	TEMPERATURE (° F.)	W.B.C.	EOSINOPHILES (%)	TOTAL DOSAGE OF DRUG (GM.)	RESULTS
C. U.	39	F	None	Yes		98.6	6,200	2	40	Slight relief
I. C.	55	M	Sinusitis	No	Mixed	Normal	10,650	7	13	No relief; nausea
C. B.	52	F		Yes	Pneumococci and streptococci	99.4	12,300	0	14	Good
A. H.	60	M	Prostatitis	Yes	*Streptococcus viridans*	Normal	9,700	1	15	No relief
V. S.	46	M	Emphysema	Yes	Staphylococci	Normal	10,800	8	12	Worse
A. C.	42	F	Hay fever	No	Pneumococci and staphylococci	Normal	6,200	4	15	Slight relief
S. B.	39	M	Bronchiectasis	No	Staphylococci chiefly	99.2	11,500	9	22	No relief
G. G.	39	F	Acute bronchitis	Yes	Mixed	101.0	19,100	14	30 (3 levels—0.91, 0.95, and 1.11)	No relief
B. B.	41	M		Yes	Streptococci and pneumococci	99.2	14,950	11	31 (level 2.94)	No relief

TABLE III

BRONCHIAL ASTHMA TREATED BY SULFAPYRIDINE

NAME	AGE (YEARS)	SEX	COMPLICATIONS	HOSPITALIZED	ORGANISMS IN SPUTUM	TEMPERATURE (° F.)	W.B.C.	EOSINOPHILES (%)	TOTAL DOSAGE OF DRUG (GM.)	RESULTS
A. H.	60	M	Prostatitis (chronic)	Yes	*Streptococcus viridans*	Normal	9,700	1	35	No relief
M. C.	53	F		Yes	Pneumococci	101	7,800	4	10	No relief; nausea and vomiting

Total number, 23; ages, 37 to 67 years; sex, 14 females, 9 males; hospitalized, 16; ambulant, 7.

In an effort to evaluate the effectiveness of chemotherapy twenty-three patients with severe asthma were selected. All were adults and their ages ranged from 37 to 67 years. There were fourteen females and nine males. Sixteen of the patients were treated at either Cook County or Wesley Memorial Hospitals; the other nine were seen at the office.

All the patients had severe asthma, and all had failed to improve by the usual therapeutic methods used in allergic cases. All but one of the hospitalized patients had been in air-filtered rooms equipped with rubber-covered mattresses and pillows. Each had received injections of 1:1,000 epinephrine subcutaneously as well as epinephrine in oil intramuscularly. Likewise aminophyllin intravenously, as well as other drugs, e.g., iodides and apomorphine, had been administered. Still other procedures had been tried in some of the patients but to no avail.

Smears of the sputum were made in most of the cases and sulfanilamide was prescribed for those in which streptococci predominated; sulfathiazole was used for both staphylococci and pneumococci. Two patients were given sulfapyridine, but nausea and vomiting limited the usefulness of this drug.

Leucocytosis occurred usually but was not extreme. Fourteen of the twenty-three patients had 4 per cent or more of eosinophiles in the blood.

TABLE IV

RESULTS SUMMARIZED

DRUG	TOTAL NO. OF CASES	IMPROVED OR GOOD	SLIGHT OR NO RELIEF
Sulfanilamide	12	3	9
Sulfathiazole	9	1	8
Sulfapyridine	2	0	2
Total	23	4	19

The three drugs were, for the most part, given in doses of 1 Gm. four to six times daily. In a few patients this was preceded by an initial dose of 2 to 4 Gm. The total dosages ranged from 10 to 58 Gm. (Tables I-IV). In three patients blood levels were done, and, despite fairly high dosage, the concentrations ranged from about 1 to 3.8 mg. per 100 c.c. This by no means equals the amount thought advisable by Keeney, but treatment was stopped in these three patients because there was no improvement.

The results of the series were, for the most part, quite disappointing. One patient was given sulfanilamide on entrance to the hospital, and her condition cleared up at once. On previous admissions she had also recovered, without the use of sulfanilamide. In three other patients there was some improvement following chemotherapy. In the other nineteen cases, however, no relief was obtained and the drugs were discontinued.

In a few cases more than one of the sulfonamide drugs was tried on the same patient. One, a severe chronic asthmatic, was given all

three, with complete failure. Three other patients received both sulfathiazole and sulfanilamide without benefit (Tables I-IV).

COMMENT

It is realized that this work is by no means as thorough as it would have been under ideal circumstances. In many of the cases, however, it was carried out when the number of patients in the hospitals was too great to permit a complete investigation. Despite the failure to obtain cultures of the sputum and more levels of the drug in the blood, the fact remains that these patients with severe chronic asthma were, for the most part, not benefited by sulfonamide compounds. It is possible, of course, that, if the drug had been administered over a much longer period of time, the results might have been more encouraging.

CONCLUSIONS

1. Twenty-three adult cases of severe intractable asthma were treated by one of the sulfonamide drugs. Sixteen of these were hospitalized.

2. Twelve were treated by sulfanilamide; nine, by sulfathiazole, and two, by sulfapyridine.

3. The results were not encouraging since only four of the twenty-three patients were improved. In the remaining nineteen there was no relief.

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DISCUSSION

DR. WALTER L. WINKENWERDER, Baltimore.—Dr. Furstenberg probably will go into this more fully than I since he has had a series of cases, but I should like to ask Dr. Unger whether he differentiated in his cases between acute infection and chronic infection. Were the patients hospitalized, or did they have fever? Did they really have evidence of bronchial or pulmonary infection?

DR. FRANK F. FURSTENBERG, Baltimore.—We too have been interested in the chemotherapy of bronchial asthma and began our study in October, 1939. At first we gave our severe chronic intrinsic asthmatic patients a therapeutic trial with chemotherapy. At the time that we began our observations sulfapyridine was the only drug other than sulfanilamide which was available, and sulfapyridine was the drug of choice. Dr. Leslie N. Gay, Dr. Nathan Herman, and I have been following these patients, and in this period some of them have been given a trial with sulfapyridine, sulfathiazole, and sulfanilamide. Our method is quite similar to Dr. Unger's. Patients who were chiefly ambulatory were given 2 to 4 Gm. a day of the drug in divided doses. We followed their bloods closely, examined the sputa before, during, and after treatment, and checked the urine frequently. We discontinued the administration of the drug at the first sign of any serious toxic manifestation.

Some of our patients had received the drug continuously for as long as three months. We have observed over seventy-five patients, but we are not ready to report our results; although we could report our failures, we are not so certain of reporting our successes as yet. At present we have gathered the data on forty-four of the patients in the treated group; five of these patients after a course of sulfapyridine or sulfathiazole have had complete or almost complete relief of asthma for a period of ten months or more. We have noted a number of patients who seemed to improve for days; then the asthma recurred unaffected by further treatment or change to another sulfonamide preparation. In the five patients with whom we had good results the improvement was immediate with almost the first dose of the drug and the patients have remained well, two of them without any asthma and the others with slight occasional asthmatic attacks which respond to a dose or two of ephedrine.

In several patients with status asthmaticus who were epinephrine fast we used chemotherapy sometimes for the want of anything better, and it seemed at times to control the asthma enough so that these patients could be treated again with epinephrine effectively.

Chemotherapy is not without danger. In twelve of the forty-four cases there has been an ominous type of toxic reaction, that is, drug fever, drug eruption, edema, a depression of the white count below 4,000, and a precipitous drop in the hemoglobin in one case.

I should like to add a word of warning about indiscriminate use of sulfonamide drugs in asthma. In three patients asthma has been precipitated or aggravated by the use of a sulfonamide preparation. Dr. Keeney has seen two patients who were receiving one of the sulfonamide preparations, sulfathiazole, I believe, and upon discontinuing the drug the asthma improved. In a case brought to my attention a child who had never suffered with asthma and was receiving sulfanilamide for scarlet fever developed asthma, but, when the drug was discontinued, the asthma disappeared immediately.

Dr. E. L. Keeney, Baltimore.—These two patients with intractable asthma who were made worse are extremely interesting, probably because of the possible explanation as to why some of the sulfonamide compounds are capable of making asthma worse. The first patient was receiving sulfadiazine, and, as the blood level rose, the asthma became progressively worse. The patient insisted that the sulfadiazine was making her worse. We were not inclined to take into consideration her advice, and it was only when she was extremely ill that we discontinued the drug. To our amazement she became well in approximately forty-eight hours. I believe I probably should have discounted this as purely a coincidence if it had not been that two weeks later I was asked to see a boy in the Brady Urological Clinic who had had a bladder operation and had received sulfathiazole. This boy had not had asthma in the past ten years. He developed first mild asthma, and then he became progressively worse. He was seen by the resident physician and was given very good treatment for intractable asthma; the only thing left for me to do when I saw him was to discontinue the sulfathiazole. He promptly got well.

When we try to explain why these sulfonamide drugs exacerbate asthmatic symptoms, two possibilities arise. One is that there is an exacerbation of symptoms due purely to the physiologic and chemical changes in the severe asthmatic patient. In these patients a carbonic

acid excess type of acidosis certainly accounts for many of their untoward symptoms. Occasionally, patients taking sulfanilamide will develop the alkali-deficit type of acidosis, and this type of acidosis superimposed upon the other should exacerbate their symptoms. These chemical changes might conceivably account for the difference between a moderately severe and a very severe type of asthma. However, the patients whom I saw were not taking sulfanilamide. Sulfadiazine and sulfathiazole do not produce an acidosis. Therefore, one must look elsewhere to find the explanation. When I discussed these cases with Dr. Long, he called to my attention the fact that he had seen several patients with an urticarial-like skin lesion following the administration of sulfapyridine and sulfathiazole. It seems to me that this same fundamental physiologic reaction which has occurred in the skin might also occur in the bronchial mucous membranes and thus account for the exacerbation of asthmatic symptoms in these two patients.

DR. R. KERN.—May I suggest a third possible mechanism in Dr. Keeney's case. At some previous date the patient may have received a sulfonamide drug and may have become sensitized. When he received it the second time, he had his allergic response. I have seen that occur in some patients.

DR. GRAFTON T. BROWN, Washington, D. C.—I have used the sulfonamide preparations on a limited number of asthmatic patients and have seen enough encouragement to lead me to believe that they should be resorted to in asthmatic bronchitis or bacterial asthma. I have observed some asthmatic patients whose attacks started with a bad sore throat. Cultures from the throat frequently reveal a *Streptococcus hemolyticus* infection, and one or two neoprontosil tablets dissolved in the mouth every four hours act both locally and systemically in aborting the infection before it reaches the asthmatic stage. The best results from the sulfonamide drugs are obtained by giving large doses over a short period of time, rather than by giving smaller doses for a longer period. Dr. Unger seems to stress smaller doses, and he did not get the optimum level of the drug in the blood which I believe one should get to obtain the best results in acute cases.

I saw one woman in severe status asthmaticus and prescribed sulfanilamide (neoprontosil) 20 grains every six hours for the first two days, 15 grains every six hours for the next three days, and then 10 grains every six hours for another seven days. The drug worked dramatically and relieved her completely of this attack. She got up and walked around, free of asthma, but later developed status asthmaticus again, and the same drug failed to relieve her. She died in this second attack. Recently I saw a child with severe asthmatic bronchitis secondary to whooping cough. She had been coughing and wheezing almost constantly for a month, and these symptoms all cleared up within a week on sulfathiazole therapy. I have also seen some chronic cases of asthmatic bronchitis that did very well on sulfonamide preparations. One patient, a man with chronic asthmatic bronchitis, who had been getting along very well on autogenous vaccine therapy, suddenly suffered an acute exacerbation of his trouble, and cultures revealed large numbers of pneumococci in his sputum. Sulfathiazole gave him prompt relief.

For *Streptococcus hemolyticus* infections, straight sulfanilamide or neoprontosil should be used; for pneumococcus infection, sulfapyridine or sulfathiazole; for staphylococcus, sulfathiazole; and for *Streptococcus*

viridans, sulfathiazole may be tried, although the sulfonamide drugs have little effect on *Streptococcus viridans* infections. In the chronic cases, I would give 10 or 15 grains three times daily for one week, and then 5 or 7.5 grains three times a day for a second week.

The sulfonamide preparations are a valuable contribution to our medical armamentarium, and we should consider their use in all cases of bacterial asthma.

Dr. Leon Unger, Chicago.—Dr. Winkenwerder asked me if I differentiated between the acute infectious type of asthma and the chronic asthma without infection. It is very difficult to do that. Dr. Brown brought up that point by saying that the drug is very useful in the infectious type or bacterial type of asthma, but it is not easy to say which group of asthma really is bacterial and which group is not. If one considers fever and leucocytosis as indicating the infectious type of asthma, then we had some cases in this group that were of that class. There were some patients who had normal temperatures and no leucocytosis. As you saw from the tables, the results in the two series were not very different.

I was very much pleased with Dr. Furstenberg's work, and I believe that he handled his cases more thoroughly than I handled mine. He had more frequent blood levels and sputum examinations before, during, and after treatment. I was glad to hear him say that he had five patients who obtained complete recovery, lasting as long as ten months or more. The fact that his patients were able to use epinephrine after starting sulfonamide drugs and were able to get the effects of the epinephrine is nothing new. If asthmatic patients do not receive epinephrine for one week, usually the epinephrine again becomes effective; that is one of the advantages of using another method of treatment, e.g., typhoid vaccine intravenously or sulfonamide compounds, in a patient receiving too much adrenalin.

In our cases there was no evidence of toxicity, e.g., rise of temperature or eruptions. There was no drop of hemoglobin or red cells in the twenty-three patients. Several patients definitely became worse after taking the sulfonamide drugs and had to discontinue the treatment. One patient, in particular, who received 30 to 40 Gm. of sulfathiazole, was made very much worse. She improved remarkably as soon as we stopped the drug and has remained well for over two months. It may be that the drug did do her good even though we counted her case as a failure at the time she was in the hospital.

I was interested in Dr. Kern's and in Dr. Keeney's remarks about sensitization phenomena. The patients had probably been sensitized by previous administration of the drug.

Dr. Brown is enthusiastic about the use of the drug in these bacterial cases. The results justify further trial in severe cases which have failed to improve on other methods of treatment.

THE USE OF BOVINE ANTITOXIN FOR THE PROPHYLAXIS OF TETANUS

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APPROXIMATELY 50 per cent of all deaths from tetanus in the United States occur in children under 15 years of age, so that the prophylaxis of tetanus is at least as important to the pediatrician as to any other medical specialist. Toxoid would appear to be an ideal agent for the prophylaxis of tetanus. There is as yet, however, no simple method comparable to the Schick test for immunity to diphtheria to determine whether or not any given child is capable of being immunized by tetanus toxoid. This is doubtless the reason why tetanus toxoid has not been used routinely by the great majority of pediatricians. Most of them apparently feel, as I do, that the physician who depends upon tetanus toxoid to protect against tetanus is practically in the same position as the physician who does not take a culture and withholds diphtheria antitoxin in the presence of a suspicious membrane in a patient who has received diphtheria toxoid for prophylaxis, but who has never had a Schick test.

Because of my particular interest in allergy as applied to pediatrics, the majority of my patients are either allergic themselves or members of families with major allergic diseases. My experience with horse serum tetanus antitoxin in these patients was consistently disagreeable. In 1933 it was brought to my attention that a bovine tetanus antitoxin was made in this country by Sharp and Dohme in Philadelphia, and since that time I have used no other preparation.

Bovine antitoxin for the prophylaxis and treatment of tetanus and diphtheria was introduced by Kraus and his associates in Buenos Aires about 1921.[1] They did this because wide experience in the therapeusis of typhus and human anthrax had shown marked freedom from reactions following the use of normal bovine serum. They demonstrated that bovine tetanus and diphtheria antitoxic serums were just as effective as similar equine preparations but that their use was to a large extent free from the disagreeable sequelae of horse serum.

Bovine tetanus antitoxin was introduced into this country in 1929. Since the original work of Kraus there has been very little published on bovine tetanus antitoxin, although in 1936 Glaser and Landau,[2] in discussing the prophylaxis of allergic disease, advised the use of bovine

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tetanus antitoxin with particular reference to the avoidance of serum reactions and sensitization to horse serum.

My experience with bovine tetanus antitoxin consists of thirty-eight cases. The patients range in age from 1.5 to 18 years, with an average of 7 years. Although I have used the preparation in adults, they are not included in this series; but it may be said that adults tolerate the serum fully as well as do children.

CASE REPORTS

CASE 1.—A boy was admitted to the emergency treatment room of the Genesee Hospital for treatment of numerous brush burns and scratches received as the result of an auto accident in which he had been dragged for a distance along the road. The attending physician advised tetanus antitoxin, and the boy was given a routine intradermal test with horse serum diluted 1:10. Immediately following this, he became cyanotic and unconscious and made convulsive movements; it was thought that he was going to die because of immediate anaphylactic shock. However, he revived after the subcutaneous injection of epinephrine hydrochloride, 1:1,000 solution, and with artificial respiration. He was kept in the hospital overnight and was referred to the allergy clinic a few days later. At this time he was tested with 1:10 bovine serum intradermally with negative results (following a negative scratch test to the same substance), and 1,500 units of bovine tetanus antitoxin were given in divided doses over a period of one hour with no difficulty whatsoever. The boy was detained at the hospital for several hours before being sent home. Two weeks later he reappeared at the clinic and reported no symptoms which might be interpreted as a serum reaction. This was the only case which was not treated in private practice and, since it was seen early in the series, impressed upon all observers the value of bovine tetanus antitoxin.

CASE 2.—A boy had had asthma since the age of 2 years. At the age of 8 years he came under treatment, which was discontinued against advice at the age of 11 years; he had been completely free from attacks during the last year of treatment, except for two instances when an adequate and easily avoidable exposure caused mild attacks. On July 4, 1940, a firecracker exploded in his hands, severely lacerating the fingers and palms. The wounds were treated by a local physician who also attempted to administer horse serum antitoxin despite a strongly positive skin test. The patient was given two injections of an unknown but small amount at half-hour intervals. One half-hour after the second injection he experienced itching about the eyes, broke out with generalized urticarial wheals, and complained of abdominal pain and difficulty in breathing. The symptoms were relieved by the administration of epinephrine hydrochloride, and the boy was brought back to Rochester. The following day he was somewhat edematous and asthmatic and suffered from nausea and vomiting. A day later he was given a scratch test with bovine serum, and this was followed by a negative intradermal test to the same substance. Fifteen hundred units of bovine tetanus antitoxin were given in divided doses without difficulty. Two days later he experienced a severe delayed reaction to the previous injections at the site of the previous skin test with horse serum and at the two sites where the small amounts of horse serum had been injected. There was no reaction whatsoever at the sites of testing and injection with the bovine serum. He was uncomfortable for a number of days from this, and, when it cleared up about six days after the original injury, he was again given 1,500 units of bovine tetanus antitoxin with no disturbing sequelae.

In three instances, because of subsequent injuries weeks to months after the first injection of bovine tetanus antitoxin, it was necessary to repeat the injection. Disagreeable sequelae of any kind were not encountered.

The personal history of the patients was known in all but one case. In the remaining thirty-seven children there was a personal incidence of approximately 49 per cent of allergic disease in the patients treated. Certainly a series of this type should be suitable for testing the safety of the parenteral injection of a foreign protein.

Of the thirty-eight patients in the series, fifteen, or 39.5 per cent, experienced some reaction to the bovine antitoxin, varying from a questionable itching at the site of injection of the serum to two instances in which there was moderately severe serum sickness. The incidence of serum reaction was divided equally between those patients with a personal history of allergy and those patients who had no personal history of allergy. The usual reaction was the appearance of a few urticarial wheals lasting from a few minutes to a few hours. In no case in this series was there a question of any immediate anaphylactic reaction to the bovine tetanus antitoxin, and in only one instance was the serum sickness severe enough so that the parents wanted the child to be seen by the physician.

Since the purpose of this presentation is to demonstrate the relative safety of bovine serum over horse serum for the prophylaxis of tetanus, it would be desirable to compare this series with a series of horse serum injections as regards complications and sequelae.

The only published series of reactions following the prophylactic injection of horse serum for tetanus in amounts comparable to the bovine serum employed in my series is that of Lyall and Murdick.[3] These authors published the results of a series of 1,000 cases in patients of all ages. Fifteen and two-tenths per cent of the patients developed generalized reactions (of which only 0.2 per cent were alarming) as compared with two generalized reactions (neither of which was immediate or alarming) or 5.1 per cent in my series. The protein content of the horse serum averaged 9 per cent; that of the bovine serum averaged 7 per cent. My series, however, is so small that the above comparison with the series of Lyall and Murdick possesses only academic interest.

It is tempting to speculate that most persons are relatively immune to bovine serum because the proteins of the serum are probably much the same chemically and immunologically as those of beef and milk and that through the ages, as man has used these substances for food, he has built up an adequate mechanism for detoxifying these proteins. However, we know that this is not true because cow milk sensitivity is the most common form of allergy encountered in infancy. The explanation doubtless lies in the observation of Wells and Osborne[4] that animals fed with

milk are not good subjects upon which to try anaphylaxis experiments with milk since they may have acquired more or less of an immunity. Incidentally, this observation probably explains the rarity of acute anaphylaxis in man following the use of milk protein for nonspecific foreign protein therapy. I have had no experience with what might happen if a milk- or beef-sensitive person were given bovine serum. I have observed one four-month-old infant who had eczema and who gave strongly positive passive transfer tests to cow's milk, beef muscle, and bovine tetanus antitoxin.

SUMMARY

1. Bovine tetanus antitoxin has been used with safety in selected persons known to give severe anaphylactic reactions to horse serum.

2. The only indication for the use of equine tetanus antitoxin occurs in those cases where the person is known to be, or can be demonstrated to be, sensitive to the bovine but not to the equine preparation. Thus far no such cases have been reported.

3. Persons undergoing an allergic study should be tested routinely with equine and bovine sera. This should be done by the scratch method only to avoid as far as possible the sensitizing of the patient to the serum. If the patient is sensitive to both sera, he should be immunized by means of tetanus toxoid, the precautions suggested by Cooke and his associates[5] being carefully observed.

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DISCUSSION

Dr. Louis Tuft, Philadelphia.—I believe that the important contribution of Dr. Glaser's paper is that it calls the attention of practitioners to the fact that there is such a thing as bovine tetanus antitoxin. Most of the serum reactions that follow tetanus antitoxin injection occur because of a failure to take the necessary precautions in doing a preliminary skin test and in determining that that person is or is not allergic. Very often the test is given in the accident wards of hospitals without any such precautions. When a positive skin test is obtained, the practitioner sometimes attempts desensitization, whereas, if he knew that bovine antitoxin was available, he could inject it, providing that the preliminary skin test was negative.

I do not entirely agree with Dr. Glaser in advising bovine antitoxin in preference to equine because, if a practitioner would take the necessary precautions, the incidence of serum reactions after horse serum tetanus antitoxin would be no greater than that after bovine. Furthermore, the antitoxin prepared at present with the newer methods has a very small

amount of the serum sickness-producing substance. The amount of bovine serum injected is three times that for equine. For 1,500 units we have to give 3 c.c. of bovine, and, if it is a severe case and we want to give 3,000, 6 c.c. is necessary, whereas the equine dose is one-third of that amount. As a matter of fact, if we examine the statistics in this small series, we find that serum reactions occurred in one-third of the persons who were treated with bovine serum. Even though the reactions were mild, they still constitute evidence of serum reactions. I maintain, therefore, that with the equine serum, if the preliminary skin test had been done, the serum reactions would not be even that great. I feel that the bovine serum should be used only in those cases that show a positive skin reaction to horse serum instead of attempting desensitization.

Finally one point that is of interest here, aside from the question of whether we should use bovine or equine serum, is the general conception that the allergic person is more likely to have serum sickness than the nonallergic. I do not believe that is true except in those patients who are sensitive to horse epithelium and horse dander. The fact that a person who does not fall in the latter group but who has asthma, hay fever, rhinitis, or eczema is not any more likely to develop a serum reaction than the normal nonatopic is borne out in Dr. Glaser's series. If we examine the group of allergic patients, we find that 8 had asthma, hay fever, or rhinitis and had no serum reaction whatever; on the other hand, there were serum reactions in six who had asthma, hay fever, or some other type of allergy, and those serum reactions were mild. It is interesting that the only severe reaction in the whole group occurred in a patient who was free of allergy. I believe, therefore, that one should not hesitate to give tetanus antitoxin or any type of serum to an allergic patient merely because he happens to have hay fever or asthma and that he is not any more readily sensitized than the nonallergic patient.

Dr. Samuel Feinberg, Chicago.—I might make one suggestion. In view of the fact that it is a frequent phenomenon that active sensitization occurs after initial horse serum injection, it is very likely this would occur after bovine serum injection, and then there would be added possible difficulty of sensitivity to meat. I believe that that is not an unlikely probability.

Dr. Leon Unger, Chicago.—In the January issue of The Journal of Allergy a case similar to Dr. Glaser's was reported. The patient had a severe reaction to horse serum. Despeciated tetanus antitoxin was used without trouble and with excellent results. When given in a case of tetanus, I believe, too, that we should stress the use of tetanus toxoid prophylactically. Some allergists have been very lax in contributing to the general comfort and well-being of their patients. Some of us are so intent on our allergy problem that we forget that our patients are human beings and must be protected as much as possible. The allergist and pediatrician should take the initiative in pushing this tetanus toxoid campaign. It has been going on effectively and has been used for some fifteen or sixteen years, especially in France. The whole French army is immunized as well as the French navy. The members of the United States Military Academy have all been immunized. Many pediatricians are using combined tetanus-diphtheria toxoid. If all patients were im-

munized with tetanus toxoid, given in two doses, we would not have to worry about whether we should use the bovine antitoxin or the horse serum antitoxin.

DR. GLASER.—As to Dr. Tuft's remarks, I believe that, with serum as well as with other allergens, too much dependence cannot be placed upon the skin tests alone. We know perfectly well that occasionally we may give relatively large doses of allergens safely in the presence of positive cutaneous skin tests and also that occasionally we may get severe generalized reactions when the skin tests are negative. I do not believe that we can be guided entirely by the skin tests in giving treatment. We must take into consideration not only the history of the patient being treated but also the history of the preparation we are using. In this respect I believe that bovine tetanus antitoxin is far better than horse serum.

There is at present no way of avoiding serum reactions, no matter what kind of serum is used. Serum sickness may occur in transfusion with similar species as, for example, from man to man, and also when blood is taken from the arm of one person and reinjected intramuscularly or intravenously into the same person. While serum sickness cannot be prevented, we can reduce its severity and possibly its incidence by the appropriate use of bovine antitoxin instead of horse serum antitoxin. We are, however, more interested in the prevention of the anaphylactic type of serum reaction than in the usual type of serum sickness, and in this case there is no question regarding the superiority of bovine antitoxin.

With respect to Dr. Feinberg's remarks, the possibility of sensitizing a patient to beef by the use of bovine serum is more theoretical than real. I feel that by the constant ingestion of milk and beef we are constantly hyposensitizing ourselves to the use of bovine serum. The form of serum which I should like to see introduced and which appears to be practical since the widespread introduction of serum centers throughout the country is human tetanus antitoxin. This can be made easily by immunizing people with toxoid, but of course in these cases the antitoxic titer of the blood of each person used as a donor of the serum must be determined.

With respect to Dr. Unger's remarks, the despeciated horse serum will doubtless prove to be of great value, but its use is still in the experimental stage. There is no question regarding the routine use of tetanus toxoid in mass prophylaxis, particularly in the case of large bodies of men, such as the army, the navy, and groups in certain special occupations. However, every reviewer on the use of tetanus toxoid concludes, that, if the patient, although he has received tetanus toxoid, is exposed to tetanus, then, in addition to another dose of tetanus toxoid, he should also receive a dose of tetanus antitoxen. If this is true, then the use of tetanus toxoid in the allergic child is almost completely unnecessary because the toxoid is used in these cases to avoid the necessity of giving antitoxin to the allergic patient.

IMMUNOLOGIC STUDIES WITH CONJUGATED RAGWEED POLLEN EXTRACTS

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THE skin-sensitizing antibody or reagin, although demonstrable in the serum of practically all ragweed-sensitive patients, is still present after specific ragweed therapy even though clinically all hay fever symptoms have disappeared. The presence of another separate antibody, a blocking substance, was described by Cooke and his co-workers[1] and is found after treatment of normal or ragweed-sensitive patients with ragweed pollen extract. Loveless[2] has differentiated these two antibodies by demonstrating that the reagin is heat labile and the blocking antibody, heat stable. It seems significant that treatment of normal persons with very large doses of ragweed extract produced no clinical symptoms of hay fever and no skin-sensitizing antibody, but only this other blocking antibody. The other unanswered facts are that desensitization is not accomplished and that clinical improvement in large series of patients rarely exceeds 80 per cent.

Although it has been established by Cooke and his co-workers[3] that the activity of the pollen extract is closely associated with the protein fraction, Grove and Coca[4] demonstrated that protein-free extracts obtained after treatment with enzymes were still reactive. Also unpublished data by Spain and Sammis from this laboratory showed that the ultrafiltrate of ragweed extract contained very little protein nitrogen and practically 100 per cent of the nonprotein nitrogen. They found that this fraction was not only skin reactive but that treatment of a series of hay fever patients with this ultrafiltrate resulted in good clinical relief. No attempt has been made in these studies to conjugate a purified fraction, but the whole ragweed extract has been used.

These facts seem to suggest that the antigen used for pollen therapy is not complete. Otherwise why should not the skin-sensitizing antibody, which is specific for ragweed, be decreased to a greater extent after specific treatment, and why should there always be a certain percentage of definitely unfavorable clinical results, even in the presence of a blocking antibody?

Landsteiner[5] introduced the term "haptene" for that portion of complex antigen which determines the serologic specificity. A haptene

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fragment of an antigen, as the determinant of specificity, is capable of reacting with antibodies produced by the injection of the whole antigen, but by itself rarely stimulates the production of antibody. Therefore, a haptene is often called an incomplete antigen. In the case of ragweed extract, the haptene fragment of antigen, as the determinant of specificity, may be only very loosely bound or absorbed by various proteins and polysaccharides of the pollen extract. The protein itself may not be the fraction concerned in active desensitization. In other words, the protein which stimulates the production of the blocking substance may be an entirely different agent from that necessary for the decrease in the skin-sensitizing antibody. By means of the diazo reaction we have attempted to link the ragweed extract with diazotized aromatic amines so that the haptene molecule may be somewhat increased with the hope of obtaining greater immunologic responses. (If linkage of the active fraction is possible, it may also prevent hydrolysis and deterioration.)

This report will deal only with experiments in which sulfanilic acid and arsanilic acid were diazotized and then linked to the ragweed extract. Studies are being continued on the direct diazotization of ragweed extract, which is then linked further with another similar ragweed extract, and also on the diazotization of a nonprotein ultrafiltrate of ragweed.

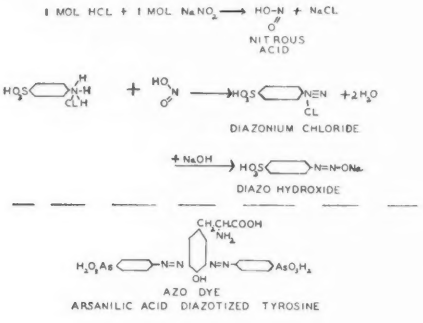

Fig. 1.

The formation of a diazo compound may be represented roughly in the manner shown in Fig. 1.

Sulfanilic acid and arsanilic acid were diazotized according to the usual methods. Since Heidelberger and Kendall[6] have shown that a true chemical interaction resulting with dye formation is possible in neutral mixtures of diazotized aromatic amines and proteins, this procedure has been followed because great variations in pH will destroy the activity of pollen extracts. Care was taken to add sufficient alkali to neutralize the acid liberated, in which case the reaction goes in the direction of complete dye formation.

Rabbits were immunized by the intracutaneous route. The conjugated ragweed was mixed in equal parts with a 20 per cent gelatin solution,

and 1-c.c. portions of the antigen-gelatin mixture were injected into two intracutaneous sites. Injections were made at three-day intervals for a total of five injection days. Animals bled sixteen days after the first injection showed precipitins in their sera by this procedure. Guinea pigs were immunized by two intraperitoneal injections with a one-day interval between doses.

Sera of rabbits immunized with the regular ragweed extract or with the diazotized sulfanilic acid-ragweed extracts failed to give positive ring precipitin tests with the diazotized compound after incubation at 37° C. for one to two hours, precipitins being obtained with the regular ragweed extract.

TABLE I

INHIBITION TESTS

0.2 C.C. SERUM	0.1 C.C. ANTIGEN	REGULAR RAGWEED		A.D.R.	SALINE
		10,000	5,000	10,000	
252	S.D.R. 2,000 U	–	–	–	–
252	S.D.R. 1,000 U	–	–	–	–
252	S.D.R. 500 U	++	++	++	–
252	S.D.R. 200 U	++	++	++	–
252	Ragweed 2,000 U	–	–	–	–
252	Ragweed 1,000 U	–	–	–	–
252	Ragweed 500 U	–	+	+	–
252	Ragweed 200 U	++	++	++	–
252	A.D.R. 2,000 U	–	–	–	–
252	Saline	+++	+++	+++	–
234	S.D.R. 2,000 U	++	++		–
234	S.D.R. 1,000 U	++	++		–
234	S.D.R. 500 U	++	++		–
234	Ragweed 1,000 U	–	–		–
234	Saline	++	++		–

252, Sensitized with S.D.R.; 234, sensitized with ragweed.

Inhibition tests were made in order to study further the interrelationship of the antigens and the antisera. No. 252 serum was obtained from a rabbit sensitized with sulfanilic acid-diazotized ragweed (Table I). Sulfanilic acid-diazotized ragweed was incubated with antisulfanilic acid-diazotized ragweed rabbit serum at 37° C. for one hour. The mixtures were then placed in an icebox for at least twenty-four hours and centrifuged. The clear serum-antigen mixtures were transferred to precipitin tubes and layered with antigen for the ring-precipitin test. These results showed that the diazotized ragweed inhibited later precipitin reaction of the antisera with regular ragweed extracts. However, sera from rabbits immunized with regular ragweed (serum No. 234) and incubated with the conjugated ragweed showed positive precipitin tests on ring test later with regular ragweed. This showed that there was no inhibition by the sulfanilic acid-diazotized ragweed with regular ragweed antisera. It is of interest to note that the inhibition reaction caused by the conjugated compound occurred only after incubation of the material

for twenty-four hours in the icebox. At first the inhibition tests were run with incubation at 37° C. for one hour, and then the precipitin-ring test followed immediately. No inhibition was shown; all tests gave positive precipitin results. This was puzzling because, since the conjugated compound had produced the antibodies, it should have been capable of reacting with them. This inhibition reaction was demonstrated easily after the incubation procedure was properly adjusted.

We feel that the negative results obtained on the regular ring-precipitin test with rabbit antiragweed sera and the sulfanilic acid-diazotized ragweed are due to the fact that certain physical characteristics of the original ragweed extract have been altered in the formation of this ragweed dye. If, as in the case with the inhibition test, conditions are adjusted to give a chance for the altered compound to react, union of the antibody with the diazo-ragweed probably will be demonstrated.

Patients sensitive to ragweed were tested with the same protein nitrogen strength of the regular ragweed extract and the sulfanilic and arsanilic acid-diazotized ragweed extracts. Closely parallel results were obtained even though part of the protein nitrogen determined in the conjugated compound was from the mono-amino aromatic acid and not from the ragweed protein. For example, the total quantity of the ragweed extract used for the diazotization contained 61 mg. of protein nitrogen but increased to 86.7 mg. of protein nitrogen after diazotization; this showed a definite gain in protein nitrogen in the conjugated extract. However, no significance can be placed on the similarity of the skin tests of the two extracts since even major variations in the potency of concentrated stock solutions cannot be accurately estimated in the low dilutions used for skin testing.

Sera from ragweed-sensitive patients were used to test the neutralizing capacity of these conjugated extracts along with regular ragweed extract. The immediate reactions and the neutralization point on retest twenty-four hours later were the same with the regular and the conjugated extract. Stull and his co-workers[7] found that formolization and acetylation reduced the ability of an extract to neutralize sensitive sera.

Arsanilic acid-diazotized ragweed also was used for sensitization of rabbits. In contrast to the sulfanilic acid-diazotized ragweed, it did react with its antisera to give positive ring-precipitin tests. It did not produce direct precipitating antibodies to arsanilic acid-diazotized timothy or to the plain diazotized arsanilic acid, but only to the arsanilic acid-diazotized ragweed. These experiments are at variance with the findings of Landsteiner and others, whose results we were not trying to duplicate since they showed, regardless of the protein attached, marked specificity to the diazotized aromatic amine, which is their haptene. In all of these experiments the specificity has remained with the ragweed fraction part which we hope to be able to show in further studies to be

of haptene nature. In this connection it is of interest to reconsider the work of Winkenwerder, Buell, and Howard,[8] who obtained, in ragweed-sensitive patients, positive skin reactions to many compounds of the purine or pyrimidine ring structure. That this may be the basic structure of the haptene involved seems suggestive to us, and studies on their diazotization with nonprotein ragweed fractions are being investigated.

We cannot assert that the conjugated extracts were linkages of the aromatic amine with the ragweed, but the definite formation of a dye compound, the large increase in protein nitrogen, and the inability of the conjugated sulfanilic acid-diazotized ragweed extract to give immediate precipitin reactions certainly suggest that such a union has taken place. These results lend no proof to the theory set forth herewith of the presence of a haptene which, if made completely antigenic through a linkage of the diazo type reaction, could then react more specifically against its antibody and cause desensitization. However, these experiments have shown that the diazo reaction has not interfered with the original antigenicity of the ragweed extract. Further conjugations with various fractions considered inactive may now be re-examined. It may be possible to complete the antigenicity of a haptene fraction which may be the specific factor related to the skin-sensitizing antibody.

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DISCUSSION

DR. WALTER L. WINKENWERDER, Baltimore.—I should like to ask several questions. In the inhibition test, were the substances used one at a time or alone and not attached to ragweed in attempting to get inhibition? Were animals which were immunized with the conjugated ragweed shocked with either one or both of the acids? When sera from animals immunized with the conjugated ragweed were employed, was either one of the acids used alone in attempting to determine whether or not positive passive transfer tests could be obtained?

DR. JOSEPH HARKAVY, New York City.—After the preparation of these compounds, was there enough ragweed antigen left to give the skin reactions obtained?

MARGARET B. STRAUSS.—We have not yet used various shocking doses and shocked animals with the related compounds, but we have immunized guinea pigs with diazotized ragweed and have tried to add the various

compounds, such as the plain sulfanilic acid diazotized by itself and also the related arsanilic acid-diazotized timothy, to the isolated uterine strip. When an animal was sensitized to the arsanilic acid-diazotized ragweed, in practically all cases, we obtained negative results with the plain aromatic compounds; this showed that we have not carried any of the specificity with this group, but it has always remained with our ragweed fraction. We have not tried sensitization with the plain aromatic nucleus without the ragweed, nor have we tried passive transfer tests with the rabbit sera obtained by immunization with the diazotized ragweed. In our study normal people with passive transfer sites with sensitive serum have not been tested with the plain arsanilic acid alone but always with our conjugated fraction. What we are trying to do now is to conjugate these compounds to a definite inactive fraction of the ragweed extract, because that would be the only way in which one can eliminate the possibility of the presence of active ragweed still in an undiazotized state. This also answers Dr. Harkavy's question. We cannot say for sure that we have eliminated all of that original ragweed antigen, but we certainly seem to have formed a new compound since we get this large increase in protein nitrogen and the altered time reactions of the conjugated compounds in the inhibition tests.

We cannot state that all of the active ragweed has been combined. That is why we feel that it would be very worth while to link up and test some of these inactive fractions because I believe that, if it were possible to link some of the simple compounds containing a purine and pyridine base with an inactive protein fraction of the ragweed extract, then we would have something significant.

STUDIES ON THE BLOCKING ANTIBODY OF COOKE IN THE TREATMENT OF HAY FEVER

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THERE is a marked discrepancy between the skin tests and the symptoms in hay fever. There is also a discrepancy between the amount of skin-sensitizing antibody and the symptoms. A good objective measure of the degree of sensitiveness is badly needed.

When in 1935 Cooke, Barnard, Hebald, and Stull[1] described the blocking antibody, it was hoped that this would prove to be the measure so much desired. In the summer of 1940, one of us (M. A. S.) conducted a study of the blocking antibody in a group of patients with ragweed hay fever. The results are comparable to those reported in a recent comprehensive paper by Sherman, Stull, and Cooke,[2] and the work is presented (1) because it does confirm the results of previous workers, (2) because it indicates the difficulties of studies of this sort, and (3) because it is another attempt to correlate the concentration of blocking antibody with the clinical end result.

The amount of skin-sensitizing antibody (''reagin'') can be titrated in two ways. The ''dilution test'' refers to the dilution of sensitive serum with saline before it is used for passive transfer. The ''neutralization test'' refers to the reactions obtained when skin sites in normal recipients are prepared with mixtures of sensitive serum plus the allergen (ragweed) in various proportions and are then tested with ragweed. The smallest amount of ragweed which inhibits the activity of the skin-sensitizing antibody in the mixture provides a measure of the antibody concentration.

Sherman and Stull[3] have found that the two measures of the antibody do not always give parallel results—the functions may be slightly different.

What Cooke and his co-workers could show was that, when ''P'' serum (obtained after treatment) was mixed with ragweed, ''neutralization'' of the skin-sensitizing antibody did not occur except when large amounts of the ragweed allergen were used. The authors concluded that treatment had resulted in the development of a ''blocking antibody'' which could inhibit the reaction between the skin-sensitizing antibody and the allergen, ragweed.

From the Allergy Clinic and Laboratory, Massachusetts General Hospital.
Presented at the Annual Meeting of the Society for the Study of Asthma and Allied Conditions, Atlantic City, N. J., May 3, 1941.

In 1937, Cooke, Loveless, and Stull[4] showed that the blocking antibody could be developed in normal persons, and the fact gave strong support to the theory that this new function was responsible for the effect of ragweed treatment. More recently Loveless[5] has shown that, whereas the skin-sensitizing antibody is destroyed by heating to 56° C. for one to five hours, the blocking antibody would resist a temperature of 56° C. for as long as twenty-four hours. She found that, when heated "P" serum was added to fresh "A" serum (obtained before treatment), this "A" was no longer neutralized by ragweed. As she put it in her discussion: "The thermostable blocking antibody in the 'P' competes successfully with the skin-sensitizing antibody in the combination with ragweed."

Meantime the findings of Cooke have been confirmed by Harley,[6] by Langner and Kern,[7] and by Schmidt and Lippard.[8] In 1940, Sherman, Stull, and Cooke[2] studied the relation between this block and the clinical end result but found no correlation. Blocking antibody was demonstrable in certain patients before treatment and was absent in other patients who were doing well under ragweed therapy. Loveless[5] also found that, whereas good results (90 to 98 per cent relief) were observed in some of her patients whose "P" serum drawn in the fall months required as much as 2,000 units of ragweed to "neutralize" the transferring capacity (skin-sensitizing antibody), her Table III shows other cases with equally good results in which only 60 units of ragweed were required. On the other hand, some patients with poor results (35 per cent or less) needed as much as 1,000 units of ragweed to neutralize, while others needed only 300 units. It is fair to say, however, that the amount of antigen required to neutralize "P" was greater than the requirement for "A" in all cases and that the ratio between the two was greater as the clinical result was better.

Our own experiments were planned to study the variations in the neutralization of the skin-sensitizing antibody before and after treatment and particularly to compare these with the clinical results.

A group of sixteen patients with typical ragweed hay fever and good skin tests were chosen. Before or at the beginning of preseasonal treatment in the months of June or July, about 15 c.c. of blood were drawn from each patient and the serum was separated. The serum was diluted 1:5 with sterile saline containing 0.5 per cent phenol. To 0.4 c.c. of the diluted serum were added 1.60 c.c. of various ragweed dilutions in series, to make a total volume of 2.0 c.c. Fresh ragweed dilutions of 1:100,000, 1:10,000, 1:1,000, and 1:100 were used plus a saline control. (The final dilutions of ragweed were therefore 1:500,000, 1:50,000, etc.) These mixtures were shaken well and allowed to stand overnight in the icebox. The mixtures were then used to prepare skin sites by intracutaneous injection into recipients, and for each patient several different recipients were used.

The recipients were patients in the allergy clinic, who had been skin tested repeatedly, and were not sensitive to ragweed, either clinically or by skin test. In fact, most of them were completely negative to all intracutaneous tests. Each recipient was injected intracutaneously with the serum-ragweed mixtures made from the blood of four to eight patients. From two to four vertical rows were placed on each upper arm, each row representing a constant amount of one patient's serum in combination with serial dilutions of ragweed, the last site containing only serum plus saline. Into each site, 0.10 c.c. of the mixture was injected, and the sites were marked with ink.

TABLE I

THE CONCENTRATION OF RAGWEED NEEDED IN THE AVERAGE RECIPIENT TO ABOLISH REACTIONS IN SKIN SITES PREPARED WITH A MIXTURE OF RAGWEED PLUS "A" OR "P" SERUM

NO.	PATIENT	SEX	AGE (YR.)	SCRATCH TESTS		"A" SERUM	TREATMENT		"P" SERUM	CLINICAL RESULTS
				BEFORE	AFTER		NO. OF DOSES	MAXIMUM DOSE (NOON UNITS)		
1	E. R.	F	37	1*		10*	13	1,000	5*	+
2	N. A.	F	55	1*	1	10*	11	1,500	3*	++
3	M. B.	F	50	1		10	12	1,000	6	++
4	N. C.	F	57	1	1	10	14	600	1	++
5	F. C.	F	27	2	1	10	4	500	3	++
6	W. W.	M	24	1		100	15	2,000	3	++
7	F. V.	F	31	1	1	100	10	500	5	++
8	A. S.	F	39	1		10	15	800	1	++
9	S. S.	M	41	5		1	15	3,000	1†	+++
10	S. C.	M	15	1		10	12	1,500	1	+++
11	J. M.	M	52	1	1	10	12	3,000	1	+++
12	J. C.	M	35	1		100	12	1,000	10	+++
13	A. T.	F	22	5		10	16	800	5	+++
14	J. B.	M	48	5	3	1	16	1,500	5†	+++
15	J. G.	M	52	5		10	11	800	3	+++
16	E. F.	F	58	0.1	1	100	14	2,000	5	++++

*The figures refer to the highest dilution in thousands which gives an appreciable reaction. Thus 1, 10, and 5, indicate a 1:1,000, 1:10,000, 1:5,000 dilutions, while 0.1 indicates 1:100.

†Decrease in "P" serum concentration. (See text.)

In each recipient, each dose of "A" serum produced an immediate reaction, with redness and swelling over an area as large as a 25-cent piece, which appeared in about ten minutes and lasted for several hours. On the next day, the recipients returned to the clinic. The immediate reactions had disappeared, and the prepared sites were tested by injecting into each 0.02 c.c. of ragweed extract in 1:1,000 dilution. The reactions were read within fifteen to thirty minutes and were recorded by freehand drawings of the wheal and erythemas as checked by measurements (in millimeters) of the average diameters.

During the season, each of the sixteen patients was treated with ragweed extract. The number and size of the doses and the intervals between them depended upon the local reactions produced and varied

considerably (Table I). At the end of the season—in late September and sometimes not until October—the patient's blood serum was obtained again, and this "P" serum (after treatment) was put through precisely the same procedure as was the "A" serum.

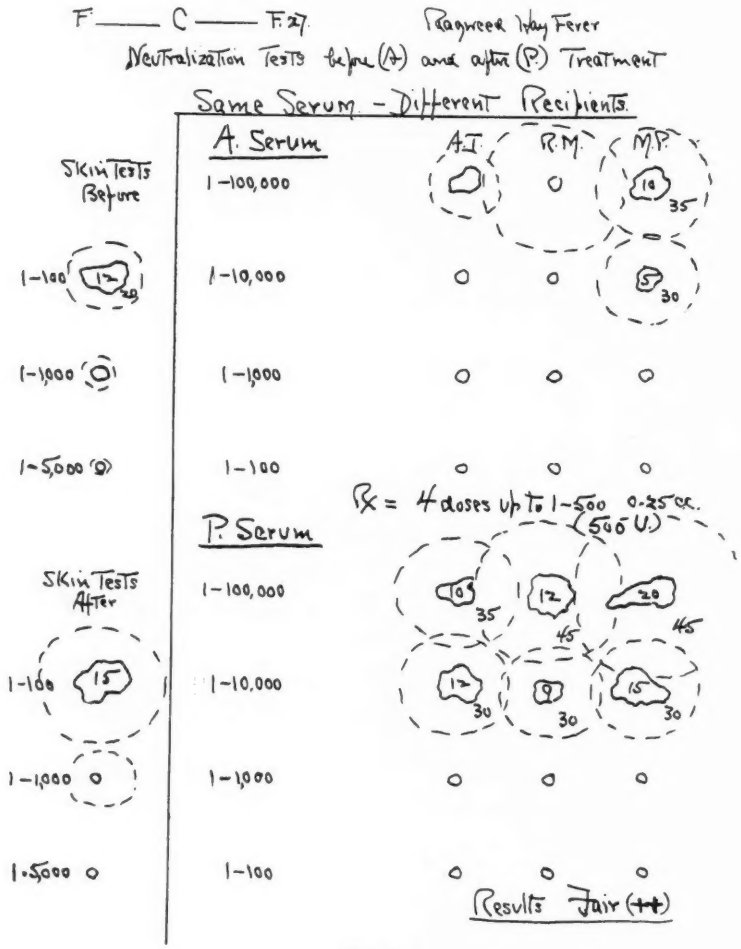

Fig. 1.

In previous work, we had been impressed with the marked variation in the behavior of different recipients, and, in order to eliminate that particular variable, the same group of recipients was used to test the "P" serum in the fall as had been used to test the "A" serum of that particular patient in the summer. To us this seemed wiser than to preserve the "A" serum until the end of the season and then to test this old "A" and the fresh "P" at the same time, as other workers had done.

Fig. 1 illustrates a typical experiment (Case 5). This woman showed good skin tests to ragweed both before and after treatment. Her "A" serum was neutralized well by the ragweed—the tests were small—but

the "P" serum sensitized the same recipients more actively, so that the addition of a 1:10,000 dilution of ragweed to the serum had no effect on its ability to transfer.*

Some variation in the recipients is noted. The results to be discussed are based on the average of these reactions.

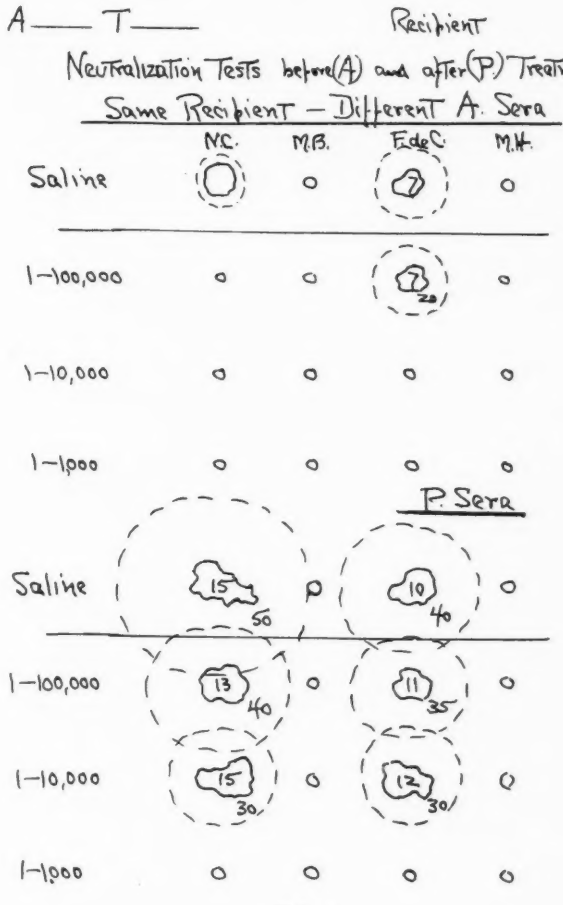

Fig. 2.

Fig. 2 illustrates the behavior of one recipient who shows well the difference between "A" sera and "P" sera. The control sites with mixtures of skin-sensitizing antibody plus saline show more of this antibody after treatment than before. Something has prevented the neutralization in the mixture, and we may assume that blocking antibody was present. The two sera which failed to react at all in this recipient were from another group of patients whose histories were typical of ragweed hay fever but whose skin tests remained negative; they contained no skin-sensitizing antibody.

*In Table I this "P" serum is listed as "3" because, in the experiment, intermediate dilutions were used and Fig. 1 is merely a summary.

The crux of the study is the end result. Table I represents a summary of the sixteen cases, arranged in accordance with the end result—from poor (+) to excellent (++++). Preliminary skin tests were positive in each case. Figures for "A" serum and "P" serum indicate the highest dilution of ragweed which caused a reaction in the prepared sites and are averaged for the several recipients. The same figures are illustrated in graphic form in Fig. 3.

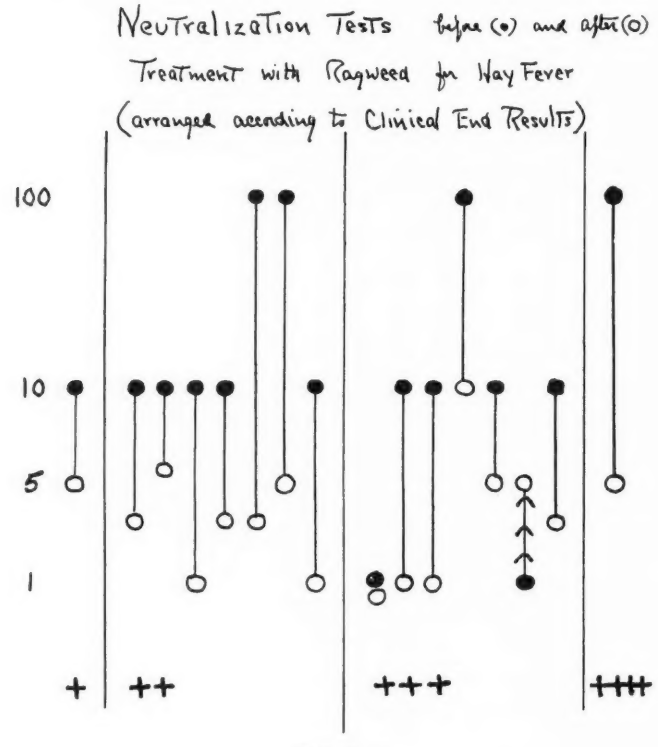

Fig. 3.

In all but two cases, an increase in the concentration of ragweed needed to abolish the reaction was observed after treatment. There was evidence of the development of blocking antibody. What is important and interesting is that the degree of difference between the tests before and after bears no relation to the end result. In Cases 6 and 7 the necessary concentration increased markedly from 1:100,000 to 1:5,000 and 1:3,000, and yet the results were only "two-plus" or "fair." In Cases 9 and 14 the end results were three-plus or good, and yet the amount of ragweed required was the same or even less after treatment than before.

If, therefore, it is true that treatment with ragweed results in a change in the neutralization of the sensitizing antibody because of the development of another function (the thermostable blocking antibody), it is also true that the amount of this new function is not proportional to the

clinical end result. The determination of the blocking antibody is not a measure of clinical sensitiveness. The clinical effect of treatment is not due to the development of this new function of the blood serum.

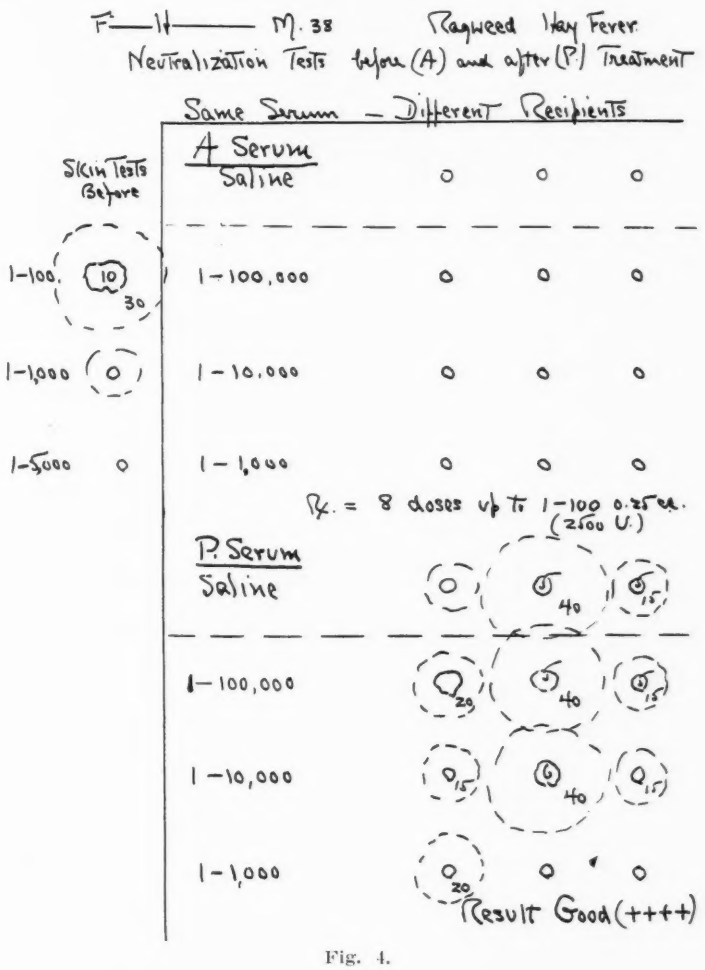

Fig. 4.

Whether our observation as described must be explained by the development of a blocking antibody or whether the differences in the reactions of "A" and "P" depend merely upon changes in the amount of skin-sensitizing antibody is doubtful. The concept of the blocking antibody depended originally upon Cooke's finding that the immediate (one hour) reaction shown by the recipient to the preparatory injection of "A" and "P" serum—ragweed mixtures—was positive for "A" but negative for "P." We, however, cannot confirm this observation. It is our experience that the injection of any and all serum mixtures in our recipients caused immediate reactions of definite extent. A more crucial test for blocking antibody might depend upon Loveless' observations on

the effect of prolonged heating as a method of isolating this particular serum function. Further study of this effect is needed.

Meantime, the findings in three of our own cases are of considerable interest (Fig. 4 is typical). In these cases, tests of the "A" serum mixture sites were flatly negative, whereas later in the season tests of the "P" serum mixture sites gave good reactions. One could say that, in the "A" serum mixture, the neutralization of the skin-sensitizing antibody by the ragweed had been complete, whereas in the "P" mixture, the blocking antibody had defeated the skin-sensitizing antibody in its contest for the ragweed. In contrast, are the controls. "A" serum plus saline failed to sensitize; the "A" contains no sensitizing antibody. "P" serum plus saline, on the other hand, sensitized well. Moreover, when ragweed was added in large amounts (1:1,000 dilution), the test became negative. The skin-sensitizing antibody had developed after treatment, and it could be neutralized.

SUMMARY

1. The results of Cooke and others have been confirmed.

2. The function of the recipient is important, and for experiments of this sort it is best to use several recipients at the same time.

3. There is no correlation between the amount of blocking antibody and the clinical end result.

4. Whether the whole conception of a blocking antibody is necessary must depend upon further study.

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263 BEACON ST., BOSTON

DISCUSSION

DR. ROBERT A. COOKE, New York City.—It is very gratifying to have this additional evidence of the existence of the so-called "blocking antibody" that Dr. Rackemann has presented.

It might be appropriate to review briefly a few points that have been developed at our clinic as a result of the later studies which have been done largely by my associates.

It has been suggested that the blocking effect is due to modification of, or to an increase of, the skin-sensitizing antibody and not a new antibody. I cannot agree with this point of view for the following reasons:

A. Quantitative passive transfer studies have shown that, although there may be an increase of sensitizing antibody in some serums after ragweed injections, there is a disproportionately large increase of antigen needed to neutralize active serum.

B. The blocking and the skin-sensitizing antibodies may be found separately or produced, even though they have not yet been separated by chemical procedure when coexistent since both appear in the so-called pseudoglobulin fraction of serum. The evidences are as follows: (1) The blocking antibody has been demonstrated in sera of normal non-sensitive persons injected with large amounts of ragweed pollen extract, but such sera did not have any capacity to sensitize normal skin to ragweed. (2) It has been established that the blocking antibody, as shown by Dr. Loveless, resists the application of heat which destroys the skin-sensitizing antibody. (3) In a publication from our clinic about a year ago we recorded observations which showed that the blocking antibody readily filtered through the human placenta and appeared in the cord blood, whereas the skin-sensitizing antibody did not appear, as had already been shown by Bell and Erickson. (4) The blocking antibody for ragweed has the capacity of combining with antigen, as Sherman has shown, although it does not form a true precipitate and thus falls in that group of reactions described by Pappenheimer and others as "soluble precipitates." There is no present ground for assuming any union of the antigen with the skin-sensitizing antibody. (5) Our studies on the blocking antibody have thus far shown that it has a very high degree of specificity for the antigen that stimulated its production, whereas the skin-sensitizing antibody of the same serum generally reacts with a large number of related and often biologically unrelated antigens.

Dr. Rackemann has brought up the important question of the relation of the blocking antibody to clinical results. Does the relation account for the immunity in treated hay fever patients? If so, we should be able to correlate the amount of blocking substance with the clinical result, but we have not been able to do this satisfactorily. When one talks about pollen injections, one is likely to regard ragweed extract as a single substance which it definitely is not. The chemicobiologic studies of Stull, Sherman, and Hampton in our laboratory have shown that there are definitely two, possibly more, proteins which we call Fraction 1 and Fraction 2. The first six sera of ragweed patients reacted on passive transfer with Fraction 1 but not Fraction 2, while the last seven sera reacted to both fractions. Thus there were two groups of ragweed pollen sensitivities. The first group, sensitive only to Fraction 1, had blocking antibody for this fraction, but, in the second group sensitive to both fractions, only one had any blocking antibody for Fraction 2. Now, if one notes the figures on clinical improvement, the patients in the second group seemed to fare just as well as those in the first group despite the fact that they had no blocking antibody for a quantitatively important fraction of the pollen to which they were sensitive. Without encroaching on what Dr. Sherman will present later today, let me call attention

to the figures for the maximum dose given. For Group I sensitive only to Fraction 1 and with blocking antibody for Fraction 1 it is readily seen that the doses reached a high level, averaging about 4,000 units. In Group II were six patients sensitive to Fractions 1 and 2, with blocking antibody for Fraction 1 but not for Fraction 2. The average for their maximum dose was less than 1,400 units. The last patient in Group II sensitive to Fractions 1 and 2 and with blocking antibody for both fractions took doses up to 7,500 units with no trouble. So our present feeling about the blocking or precipitating antibody is that its presence has to do with the capacity of the patient to take sizable and increasing doses without untoward local or general reactions. This would seem to be its function rather than to afford clinical immunity to hay fever.

It has been very pleasing to have this confirmation from Dr. Rackemann's clinic about the presence of the blocking antibody. I have reviewed the reasons for my belief in its existence separate and apart from the skin-sensitizing antibody, and my feeling is that it has not yet been proved to be the responsible agent of clinical immunity resulting from treatment but that its presence facilitates the increasing dosage and lessens the chances of undue local and general reactions during treatment.

ABORTION AFTER GRASS POLLEN INJECTION

NATHAN FRANCIS, M.D.
ROCHESTER, N. Y.

CRAMPLIKE pain in the lower abdomen with scant vaginal bleeding associated with a constitutional allergic reaction was first reported by Cooke[1] in 1922. He referred to two patients with immediate reactions, including asthma, coryza, urticaria, and menstrual flow coming three hours after injection and lasting one day. He strongly recommended caution in the treatment of pregnant women because of the possibility that abortion may be induced.

Kahn[2] in 1928 reported a case of a woman in the first month of pregnancy who complained of excruciating pain in the lower abdomen twenty minutes after receiving an injection of grass pollen. She described the pain as similar to labor pains, but more steady. There was no bleeding, and the symptoms subsided by the following day.

Robinson[3] in 1929 reported a patient with uterine pain as a part of a constitutional reaction in which there was no bleeding.

Such constitutional reactions may end with abortion, a case of which is presented.

CASE REPORT

A white female, aged 34 years, was admitted to the Rochester General Hospital with the chief complaint of vaginal bleeding. About two hours after receiving an injection of grass pollen (Nov. 9, 1939) the patient began to have hay fever which was the most intense that she had ever experienced. Soon thereafter she began to have cramps in the lower abdomen with spotting. Moderate bleeding continued until three days later when she had a profuse vaginal hemorrhage and was admitted to the hospital.

She had had an appendectomy in 1933 and a dilatation and curettage in 1938 for irregular vaginal bleeding of 1.5 years' duration. The pathologic report at that time was that of hypertrophic endometritis. Her menstrual history since then had been normal, with the last period in July, 1939. She had attended our allergy clinic since 1934 for treatment of grass pollenosis with good results from preseasonal injections until 1939 and perennial treatment thereafter. Her father has hay fever.

The physical examination showed that the abdomen was tender in the left lower quadrant. The uterus was palpated 3 fingers above the symphysis pubis. The vulva was covered with fresh and clotted blood. A fetus 9 cm. long was present in the vagina.

The patient began to bleed into the uterus soon after entry and went into mild shock. She was given acacia, fluids, and 650 c.c. of whole blood. Emergency dilatation and curettage were done. The postoperative course was uneventful with discharge on the seventh hospital day.

From the Allergy Clinic of the Rochester General Hospital.
Presented at the Annual Meeting of the Society for the Study of Asthma and Allied Conditions, Atlantic City, N. J., May 3, 1941.

The patient was moderately sensitive to grass pollen antigen. We had no difficulty in increasing the dose of injections to give her adequate protection. The maximum dose was 0.60 c.c. of a 1:250 extract on June 29. Then the dose was decreased, as is our custom, during the season through which the patient is treated, and it was again reduced in August because the patient remained away from the clinic for a period longer than four weeks.

The patient had very few symptoms of hay fever during the season when she was expected to have them, and she told us that she had had as good a year as she had previously experienced. The abortion occurred on Nov. 9, 1939, with a dose of 0.35 c.c. of a 1:250 extract. This dose was definitely below her highest.

We make our own extracts according to the method of Coca. Our stock solution is 1:50 which we prepare two or three times yearly. Grass solution contains equal parts of timothy, orchard grass, June grass, and red top. We use the perennial plan of treatment almost exclusively.

The incidence of the constitutional reaction in our experience has been less than 0.5 per cent. In 1940 we gave 3,106 injections with fewer than fifteen constitutional reactions.

An interesting observation was the leucopenia because the patient was receiving no medication which is known to depress the leucocytes. The white counts on the second and fifth hospital days were 3,900 and 3,800, respectively. Widal and others regard the drop in the total white count as one of the most important changes in the blood which may occur in the course of allergic conditions.

SUMMARY

1. Since the symptoms of severe hay fever came when no grass pollen was in the air, it was assumed that the sudden hay fever was due to the rapid absorption of the injected grass pollen into the systemic circulation.

2. Since the cramps in the lower abdomen and vaginal bleeding came on the same day and were followed by abortion three days later, it is likely that the abortion and the constitutional reaction were related.

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DISCUSSION

Dr. Albert Vander Veer, New York City.—While I have seen possibly not more than one or two miscarriages following a constitutional reaction, I have observed a number of cases in which a woman has had abdominal and uterine cramps that, had she been pregnant, would undoubtedly have led to abortion. Therefore, we exercise extreme caution with a patient who is pregnant.

Every case has to be treated individually. For instance, if the patient is one whom you have treated for several years, you have some general idea of the amount of extract she can take. If she is a new patient, you may not know much about her normal dose. In any event, we try to

underdose the patient rather than give the regular amount. It depends somewhat on when the baby is expected. If, for instance, the patient has ragweed hay fever and expects a baby in July, then it is fairly simple to give small doses until the baby is delivered, and then very rapidly increase the dose from the middle or end of July until the beginning of the season. The doses may be increased at short intervals so that by the time the season is due the patient will have enough to protect her. Of course, you can refuse to treat at all, but, if the patient has hay fever complicated by asthma, then that in itself may bring on a miscarriage. The treatment depends somewhat upon how many children the patient has had. If she is a young woman with one or two, there is less need for worry than there would be if she were having her first pregnancy. One of my patients, a woman, 40 years old, has been married for eight years and is having her first pregnancy. We have to be extremely careful in a case like that. I should say that we try to underdose such a patient, and, if we can get up to one-half of the regular dose, we can at least take the edge off the hay fever and prevent asthma. This may not be a very good result, but at least it will be on the safe side.

There are two points I should like to make about the case presented. In the first place, I believe that with perennial treatment we are more likely to get sudden unexplained constitutional reactions than with weekly treatment. With the perennial treatment frequently we see patients who seem to become saturated and have a sudden constitutional reaction after a dose no larger, sometimes smaller, than they ordinarily have carried. When we treat a patient weekly, we get a little idea from the local reaction and from the symptoms about whether a dangerous dose is being approached.

In the second place, I noticed that this patient always received adrenalin along with the injections. The adrenalin slows up the absorption, and the constitutional reaction may come after the patient has left the office and the physician cannot do anything about it.

I believe that we must be very cautious about pregnancy. In this particular case the patient was through her season before her baby was delivered. Personally, I would have stopped treatment since her hay fever was all right.

Dr. Selian Hebald, New York City.—Dr. Francis stated that the grass extract used in his clinic was made two or three times a year. How old was the extract that produced the constitutional reaction?

Dr. Francis.—The extract was about 4 or 5 months old.

Dr. Warren T. Vaughan, Richmond.—It seems to me that there are other factors to be taken into consideration in the protection of the pregnant woman. Dr. Vander Veer has outlined the specific points as far as pollen treatment is concerned, but often there are other allergic factors that should be taken care of as a matter of protection. The patient to whom I am referring was not pregnant, but such a situation might arise. A woman was having perennial treatment and was receiving 0.20 c.c. of 1:50 extract every two weeks. She was also allergic to chocolate and pork. Her husband was a physician. One night for the first time she broke her diet. She ate a hot dog and a cake of chocolate. One-half hour later her husband gave her exactly the same dose he had been giving

her every two weeks, and for the first time she had a constitutional reaction. Those additional factors that might play a part and might tip the balance would be well considered in the protection of a pregnant woman against possible abortion.

DR. HOWARD OSGOOD, Buffalo.—I should like to ask Dr. Francis and Dr. Vander Veer how many pregnant women they have carried through constitutional reactions without abortion. It does happen, as shown by the following experience several years ago. The case was that of a young woman who was quite sensitive to grass pollen, had had preseasonal treatment, and felt very well. She was seven months pregnant. On one visit by mistake she was given several times the dose of grass extract that she should have had. She left the office and was back again in fifteen or twenty minutes with a constitutional reaction—severe generalized urticaria, and erythema and marked asthma. I gave her 0.5 c.c. of adrenalin, applied a tourniquet proximal to the injection, and quieted her as well as I could psychologically. She came through perfectly all right and now has a fine baby. She also returned in the succeeding years for continued treatment.

DR. GEORGE L. WALDBOTT, Detroit.—There are many questions which confront us when we treat pregnant allergic patients. The patients want to know not only whether or not the injections will interfere with pregnancy, but also if they are likely to harm the baby after birth. Is it safe to have an anesthetic during confinement, and which anesthetic should be used? How should the patient be managed if hay fever and asthma symptoms develop during confinement? Unfortunately, we cannot answer these questions but can discuss only the impression gained from clinical experience.

We should be aware of the possibility of inducing spastic contraction of the uterus and subsequent abortion with overdoses of pollen extract. But if we proceed cautiously, I feel that such accidents can be avoided. I do not consider pregnancy a contraindication to hay fever treatment. As far as the effect of hay fever treatment on the baby is concerned, I believe that it is equally as protective to the baby as to the mother. Hospitalization for confinement affords an excellent opportunity for rendering patients with hay fever or pollen asthma symptom free for the season by cautiously giving increasing doses of pollen extracts daily or even two to three times a day.

We should be cautious about the choice of an anesthetic and the administration of drugs during confinement. I know of two patients who, shortly after confinement, developed rather severe shock which could not be explained by the obstetrician and which had the earmarks of allergic shock. It may have been caused by morphine or the anesthetic to which sensitivity existed. In one patient, the administration of aminophyllin controlled the situation very promptly.

DR. H. HUBER.—About thirty minutes after I gave one of my 18-year-old patients a pollen extract treatment, she had a mild constitutional reaction accompanied by menstrual-like cramps and menstrual flow midway between her regular menstrual periods. She afterward reported that she had had menstrual-like pains after three or four previous treatments but had had no other symptoms. Another patient, aged 35 years,

aborted a three months' pregnancy about four days after having a mild constitutional reaction consisting only of itching of the hands and feet. Since having these experiences, I have not knowingly given a pregnant patient a pollen treatment by intracutaneous or subcutaneous injection. In spite of all precautions with the regular or with less intensive treatment, constitutional reactions may occur, and I do not believe that one should take that chance with any pregnant patient. In 1934 I pointed out that about 40 per cent of pregnant patients will go through a pollen season with little or no symptoms without having any specific pollen treatment and that the majority of patients can be satisfactorily protected by applying pollens on the skin, as for scratch testing, once a week. Constitutional reactions from such a method are rare, and in my experience this method has been very satisfactory. A paper read at this meeting earlier in the day gives more evidence on the acquisition of immunity by applications to the skin.

STUDIES IN EPINEPHRINE

Recovery of Epinephrine Injected Intravenously and Subcutaneously

Stearns S. Bullen, M.D., and Walter R. Bloor, Ph.D.
Rochester, N. Y.

AN EXPLANATION of certain epinephrine effects, in terms of the epinephrine content of the venous blood, was obtained in the course of a study of the procedures necessary for the measurement of epinephrine in the blood by a chemical method.

The method[1] used is a modification of the procedure described by Whitehorn[2] in 1935 and modified by Shaw[3] in 1938. It depends upon the measurement of the blue color produced by epinephrine on a molybdate-arsenate reagent in acid solution sensitized by sulphite. Because considerable color in the blank is unavoidable, the blank is used as a reference solution; the measurement of the color is made with a photoelectric colorimeter which automatically corrects for the blank.

There are substances, other than epinephrine, in the blood which produce color with the reagent as used, and consequently correction must be made for them. These substances are alkali stable, whereas epinephrine is quickly and completely destroyed by alkali. Therefore, if the values obtained are the same before and after alkalinization, one can be sure that no epinephrine is present.

By this method, amounts of epinephrine of the order of 0.02 microgram can be measured within about 5 per cent error; that is, the presence of an amount of epinephrine as small as one part in one billion can be detected.

To check the accuracy of the method, various known amounts of commercial epinephrine in aqueous solution were measured. Because epinephrine is the only substance present which produces the blue color with the arsenomolybdate reagent, its measurement is relatively easy. The results obtained with samples of commercial epinephrine solution are given in Table I.

Portions of Samples 1 and 2 (Manufacturer 1) which had been opened and then kept in cork-stoppered bottles for about a year were found to be brownish in color and to have epinephrine values of 0.72 mg. per cubic centimeter (Sample 1) and 0.7 mg. (Sample 2).

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When the method was so standardized that consistent results could be obtained in the measurement of epinephrine in aqueous solutions, known amounts of epinephrine were added to trichloracetic acid extracts of dog's blood. Recovery of the epinephrine was practically complete (95 per cent, or more, in agreement with the results of Shaw).

TABLE I

MANUFACTURER NO.	SAMPLE NO.	EPINEPHRINE (MG. PER C.C.)	
		FOUND	CLAIMED
1	1	1.10	1.0
1	2	1.00	1.0
1	3	1.06	1.0
2	1	0.87	1.0

By the present procedure, recovery of epinephrine added to freshly drawn dog's blood was as complete as from blood extracts.

As might be expected, epinephrine injected into the circulation of a living animal can be recovered with less ease. Knowing that there is considerable variation in the amount of color-producing substances in the blood of different animals, we felt that more nearly comparable results could be obtained by using the same animal many times as the test subject rather than by doing a few experiments on each of many animals. Three dogs which had lived for two to four years under standardized conditions in the animal house were used. They were kept in individual cages, and their diet varied little from week to week. Blood samples had been withdrawn from them repeatedly, so there was a minimal amount of excitement. The dogs were used in rotation over a period of six months. Blood volume determinations were made by the brilliant red vital dye method.

A measured amount of commercial epinephrine solution of known epinephrine content was injected into one jugular vein after a preliminary sample of blood had been removed. Samples of blood were withdrawn from the other jugular vein from forty seconds to two minutes later and again after five minutes and twenty minutes. These blood samples were at once mixed with trichloracetic acid, and the measurement of the epinephrine was made promptly. All measurements were made by the same person (Bloor).

The heart rate of the dogs was used as the indicator of epinephrine activity. This rate followed a fairly constant pattern in the three dogs during repeated experiments. Within about thirty seconds after the injection, there was a sharp fall in rate to about one-half the original level, with a gradual return to nearly the original rate within five to twenty minutes. However, in one animal in which the heart had followed such a pattern repeatedly, and with the same dose of epinephrine which had been used before, there was on one occasion a sharp rise in rate in the first minute after the injection (160 to 250) followed by a

TABLE II

RECOVERY OF EPINEPHRINE INJECTED INTRAVENOUSLY

DOG NO.	EPINEPHRINE		BEFORE INJECTION	1 MIN. AFTER INJECTION			5 MIN. AFTER INJECTION			20 MIN. AFTER INJECTION		
	TOTAL AMOUNT (γ)	AMOUNT PER C.C. OF BLOOD (γ)	C.U.* PER C.C. OF BLOOD	TOTAL C.U. PER C.C. OF BLOOD	EPINEPHRINE C.U. PER C.C. OF BLOOD	% EPINEPHRINE RECOVERED	TOTAL C.U. PER C.C. OF BLOOD	EPINEPHRINE C.U. PER C.C. OF BLOOD	% EPINEPHRINE RECOVERED	TOTAL C.U. PER C.C. OF BLOOD	EPINEPHRINE C.U. PER C.C. OF BLOOD	% EPINEPHRINE RECOVERED
40-56	450	0.365	420	460	40	11	500	80	22	420	0	0
			480	480	0	0	720	240	66	400	0	0
			520	760	240	66	720	200	55	600	80	22
	250	0.2	480	485	5	0	485	5	0			
	500	0.4	250	310	60	15	360	110	22	246	0	0
40-59	250	0.14	360	480	120	86	460	100	71	390	30	21
			240	300	60	44	240	0	0	370	130	92
	500	0.28	340	360	20	7	250	0	0	230	0	0
			224	228	0	0	224	0	0	230	6	0
			250	340	90	32	340	90	32	260	10	0
37-104	500	0.23	460	420	0	0	460	0	0	520	60	26
			230	300	70	30	230	0	0	230	0	0
	750	0.345	380	460	80	23	420	40	12	420	40	12
			400	530	130	38	440	40	12	440	40	12
Av.						25			21			14

*C.U. = Color unit = amount of color produced by 1 billionth gram of epinephrine in the arsenomolybdate reagent, as suggested by Raab.[4]

fall to one-half the original level with a gradual return to this level in twenty minutes. We interpret this as evidence that we must expect varying pharmacologic responses in biologic machines even under standardized conditions such as existed in these dogs.

Because there is much variation in the amount of color-producing substances in samples of blood from the same dog at different times, it is necessary to have a preliminary sample as a control for each experiment. The values for the color-producing substances in the blood drawn before the injection of epinephrine represent substances which are alkali stable and, therefore, not epinephrine. Hence, it seems proper to conclude that there is normally no epinephrine in the peripheral venous blood of dogs. (At least, if epinephrine is present, the amount must be less than one part in a thousand million, which represents the limit of the method.) From observations not included here, the same conclusion holds good for the peripheral venous blood of human beings. This finding is to be expected, because, if epinephrine were present in the blood stream in an amount substantially greater than one part per billion, various normal processes would be disturbed; for instance, intestinal peristalsis would be inhibited.[5]

The color values above the preinjection level represent epinephrine. The greatest amount of epinephrine was recovered from the blood coincident with the greatest epinephrine effect as indicated by the change in heart rate, with an average recovery of 25 per cent one minute after injection. The quantities recovered varied from nothing, or an extremely faint trace, to 92 per cent. The average recovery fell to 21 per cent five minutes after the injection and to 14 per cent at the end of twenty minutes. In one-half of the experiments, no epinephrine was recovered in the twenty-minute samples. In three experiments, no epinephrine was found in one or the other of the first two blood samples after the injection of the epinephrine, although there was good recovery (60 to 92 per cent) in the others. We are unable to explain this discrepancy.

In one experiment, 1 c.c. of normal physiologic salt solution, containing 0.5 per cent chlorbutanol and 0.1 per cent sodium bisulfite (the solvent of the commercial epinephrine), was injected into the dog's jugular vein. There was no alteration in the heart rate, nor was there any change in the amount of color produced in the reagent.

In another series of experiments on the same dogs, measured amounts of commercial epinephrine solution of known epinephrine content were injected under the skin after the removal of a preliminary blood sample from the jugular vein. Further blood samples were withdrawn at intervals of five, ten, and twenty minutes. The measurement of the epinephrine content was made in the same way as before. As would be expected, there was much less alteration in the heart rate following subcutaneous injection than after intravenous injection. The change in

TABLE III

RECOVERY OF EPINEPHRINE INJECTED SUBCUTANEOUSLY

DOG NO.	EPINEPHRINE		BEFORE INJECTION	5 MIN. AFTER INJECTION			10 MIN. AFTER INJECTION			20 MIN. AFTER INJECTION		
	TOTAL AMOUNT (γ)	AMOUNT PER C.C. OF BLOOD (γ)	C.U. PER C.C. OF BLOOD	TOTAL C.U. PER C.C. OF BLOOD	EPINEPHRINE C.U. PER C.C. OF BLOOD	% EPINEPHRINE RECOVERED	TOTAL C.U. PER C.C. OF BLOOD	EPINEPHRINE C.U. PER C.C. OF BLOOD	% EPINEPHRINE RECOVERED	TOTAL C.U. PER C.C. OF BLOOD	EPINEPHRINE C.U. PER C.C. OF BLOOD	% EPINEPHRINE RECOVERED
40-56	750	0.61	246	200	0	0	246	0	0	246	0	0
			240	246	6	0	240	0	0	240	0	0
			420	420	0	0	500	80	13	440	20	0
37-104	750	0.35	224	204			200	0	0	200	0	0
			380	420	40	11	380	0	0	370	0	0
			188	246	58	16	188	0	0	188	0	0
40-59	750	0.425	280	400	120	27	380	100	23	340	60	14
			240				264	24	5	240	0	0
			240	224	0	0	248	8	2	246	6	0
Av.						7			5			1.5

heart rate followed the same pattern as that produced by the intravenous injection of epinephrine, with a drop in rate within the first five minutes, followed by a gradual return to the preinjection rate within ten to fifteen minutes.

The amount of epinephrine recovered from the blood was much less than that obtained after the intravenous injections, with an average recovery of 7 per cent five minutes after the injection and 5 per cent and 1.5 per cent after ten and twenty minutes, respectively. In all except one experiment no epinephrine, or only a very faint trace, was found in the twenty-minute samples.

COMMENT

Epinephrine solutions which have turned brown as the result of aging or exposure to light and air produce much less pharmacologic effect than do fresh solutions. In the aged samples studied, the epinephrine content was reduced about 30 per cent; this probably parallels the loss of pharmacologic activity.

In cases of status asthmaticus, epinephrine usually gives relief for only a few minutes. This conforms to the observation that epinephrine injected intravenously or subcutaneously either disappeared from the blood or was very greatly reduced in amount at the end of twenty minutes. It is probable that the disappearance of the epinephrine is the factor terminating the short period of relief, because it has long been known[6] that, so long as epinephrine is in contact with the tissues on which it exerts its main action, the stimulation will continue and that the tissues will not give way to "fatigue" or depression, no matter how much epinephrine is used. An excellent example is the long-continued action on blood vessels seen in the area of blanching in the skin surrounding the site where epinephrine has been injected.

By contrast, it is well known that a proper dose of epinephrine gives either complete relief or relief for several hours in the ordinary case of asthma. The explanation for this seems to be that the organs of the body have a normal and strong tendency to work together in an orderly and helpful manner. When a disturbance of function, such as an attack of asthma, occurs, the tendency toward a return to normal is still present. Of course most asthmatic attacks cease even if no treatment is given. It seems not unreasonable to assume that a therapeutic dose of epinephrine, although it may be present in the tissues for only a few minutes, aids greatly in the return to the normal state, which, once established, tends to persist.

SUMMARY

1. Epinephrine in aqueous solution and in blood can be measured chemically in amounts of the order of 0.02 microgram, within an error of 5 per cent.

2. Samples of commercial epinephrine solutions which had become brown with age were found to have lost nearly one-third of their epinephrine content.

3. Various amounts of epinephrine added to trichloracetic acid extracts of dog's blood and to freshly drawn dog's blood were recovered practically completely.

4. Following the injection of epinephrine solutions of various strengths into the veins of dogs, epinephrine was recovered from the venous blood in amounts from 0 to 90 per cent, with an average recovery of 25 per cent one minute after injection, 21 per cent five minutes after injection, and 14 per cent twenty minutes after injection. One-half of the blood samples showed no epinephrine at the end of twenty minutes.

5. After epinephrine solutions were injected subcutaneously in dogs, epinephrine was recovered from the venous blood in amounts varying from 0 to 25 per cent, with an average recovery of 7 per cent five minutes after injection, 5 per cent ten minutes after injection, and 1.5 per cent twenty minutes after injection. In all except one experiment, no epinephrine was found twenty minutes after the injection.

6. Normally, in dogs and human beings there is no epinephrine in the peripheral venous blood (or at least less than one part in a thousand million).

7. On the basis of the chemical findings, some of the commonly observed therapeutic effects of epinephrine are discussed.

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DISCUSSION

DR. E. L. KEENEY, Baltimore.—I believe a more satisfactory method of measuring the effect of the duration of the absorption of epinephrine would be to follow the cardiovascular response to epinephrine rather than the symptomatic relief of asthma. In 1920, Dr. Paul Clough published fine observations on a large group of patients in whom he demonstrated that epinephrine hydrochloride varies considerably in the rate of absorption. The initial response occurs from five to fifteen minutes after injection, and the blood pressure rise may persist for thirty to ninety minutes.

DR. LEON UNGER, Chicago.—We have all had the experience of using 1:100 epinephrine spray and of observing that it turns brown. I should like for Dr. Bullen to tell us about that. The patient often states that, when epinephrine turns brown, it loses its efficiency to a certain extent. Is that comparable to a 1 to 1,000 solution?

DR. HOWARD OSGOOD, Buffalo.—I should like to ask Dr. Bullen if he has made any clinical studies on patients to determine the amount of epinephrine in the blood after the administration of the drug therapeutically. This would be interesting particularly in patients who have taken 1:100 epinephrine by spray. We used to believe that this drug, when taken by mouth, is not effective, or at least not enough is absorbed to produce therapeutic results. I am sure that many of you have seen patients who, when they have used 1:100 epinephrine by inhalation in excessive amounts, have absorbed enough either from the respiratory mucosa or from swallowing to increase the blood pressure. Certain patients become panicky over their asthmatic paroxysms. A woman in the forties had a normal systolic blood pressure of 135, a diastolic around 75 or 80. On one occasion she was discharged from the hospital after a particularly severe bout of asthma. She was instructed to use the 1:100 epinephrine spray judiciously before a paroxysm became too severe. Four or five days later she was readmitted with severe asthma, and it was found that her systolic pressure was over 200, with a diastolic pressure of 110. From the nurse in attendance outside, it was learned that she had used the spray every fifteen to thirty minutes while awake. When the spray was discontinued after the second admission and aminophyllin was given intravenously to control the dyspnea, the blood pressure returned to normal in a day or two. I believe that the severity of her dyspnea this time was aggravated by the additional labor of the heart produced by the elevation of her blood pressure and that this elevation was due to the absorbed epinephrine.

DR. BULLEN.—This work was done primarily from the chemical point of view. My part in it was really very small. It was mainly Dr. Bloor's work. We are trying to get a method which could be used repeatedly because the method has to be used by the same person and requires considerable skill in order to get the same results on two successive days. Therefore, most of the time the work has been along that particular line, and it is for that reason that a more careful record of the cardiovascular response in these animals was not made. It was largely because of the desire to make the chemical side of the study complete. We have had the opportunity to do very little with human beings. Since this method has been sufficiently advanced to make us feel that it could be used with satisfactory results, it so happened that our hospital had had a great dearth of patients who needed large doses of epinephrine. When you consider that we are using dogs which weigh about 30 pounds and are giving them 1 c.c. of 1:1,000 solution of epinephrine, and the very small proportion of that we were able to recover, you can see what a very much smaller proportion we probably could recover in a 120- to 150-pound human being.

POLLEN STUDIES

A Contrast Color Method of Examining Unstained Pollen Grains

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THE study of pollen grains has intrigued botanists and physicians from the earliest times. In these tiny, complex cells are stored all of the potentialities of awe-inspiring trees, colorful flowers, and obnoxious weeds. Our very existence is dependent upon the growth of plant life of which pollen is an essential phase. The structure of many pollens is beautiful. The sculpture and architecture of these cells reveals a beauty of form which equals any of nature's most glorious creations. Within these grains lies the mystery of life itself. These elements of life, when joined to the reproductive cell of the ovum, will reproduce in all of its entirety the plant from which it came. The active element in fertilization is the intine or living internal cellular structure. The exine or protective covering is responsible for the sculpturing or architectural appearance of the grain. In this protective covering we find designed some of the structural characteristics which link each grain to the species of its origin. The consistent appearance of the forms of this covering makes it possible to classify and identify them according to their derivation. Pollen grains from different plants vary in a general way so that the plant family from which they come can usually be identified by examination of the pollen under the microscope.

In 1812 Sprengel distinguished between anemophilous or wind-loving pollen and entomophilous or insect-loving pollen. The insect-loving pollens, which are more beautiful than the wind-borne pollens, are sticky and relatively heavy. The pollen of wind-borne plants is light. These grains literally cover the earth during their season of pollination. It has been known for over 100 years that these abundant wind-borne pollens are the chief cause of the symptoms of hay fever. It is estimated that at least one million tons of ragweed pollen alone are produced in the United States each season. The correct identification of pollen grains is of great importance in making accurate pollen counts. Thus any method of making the identification of pollen easier will be of benefit in the study of hay fever.

Many valuable methods of studying pollen grains have been developed, and I present the following method as a simple and improved

From the Allergy Clinic of Dr. Aaron Brown, New York University College of Medicine.

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procedure. Pollen grains have been studied in two general ways. One is in the natural, dry, unexpanded state, and the other is in a moistened, expanded state. When pollen is prepared dry, in oil, or on vaseline, it tends to retain its original, external, individual shape. Pollen examined in this unexpanded shape best reveals the architectural design of the exine or protective covering. Thin, depressed areas in the exine called furrows create variations in the form of individual pollen grains. When moistened, the grains become expanded and lose some of their natural characteristics. The moistened, expanded grains become more nearly spherical, and the furrows become shallow or nonexistent. The pollen thus loses its individual structural appearance in any method of preparation which expands the grains. Thus as a basis for the method here described, only dry unexpanded pollen was used so that the pollen picture would be more nearly characteristic.

According to Wodehouse, pollen in the dry state cannot be observed to advantage in magnifications greater than 200 diameters. Magnifications greater than 200 diameters result in a loss of detail called "empty magnification." This empty magnification which gives a blurred, out-of-focus picture, is obvious in pictures of pollen in standard works on allergy. In order to compensate for this loss of detail associated with greater magnifications, it is necessary to use certain staining agents. In the simple method here developed clear detail was obtained with a magnification of 750 diameters. Color contrast was secured without using any staining agents whatsoever. An excellent method of staining has been described by Wodehouse. By the use of methyl-green glycerin jelly the exine is stained and thus can be differentiated from the intine. This method, which is in standard use, expands the grains, and thus some of the architecture of the pollen grains is lost. A number of other staining methods are also commonly used. Some methods distort the pollen to a marked extent. Some stains are not permanent. All methods require time, skill, and patience for success. In the method here used, detail, by means of color, was secured by the use of a color contrast filter.

The use of color contrast filters in microscopy was first described by Rheinberg, and the filters are known by his name. The principle of color contrast filters is similar to that of dark-field illumination (Fig. 1). Instead of a black metal disk in the condenser of the microscope as in dark-field illumination, a transparent gelatin disk is inserted. After some experiment it was found that this disk should have a central color of blue. Around the periphery of this blue disk was placed a contrasting ring of another color, and experience showed that this peripheral ring should be of a color similar to the natural orange-yellow of the pollen grains. Thus when a peripheral ring of yellow-orange was used, a good color contrast was obtained with the blue cen-

tral disk as a background. By this method the pollen grains appear brilliantly contrasted in yellow against a blue background. The external form is easily observed, and much of the structure of the cellular contents is visible. No staining of any kind is required. The Eastman filter 63 has a green center with colorless periphery; filter C4 has a blue center with colorless periphery; and filter 21 has an orange periphery with colorless center. Filters 63 and C4 are used interchangeably in conjunction with 21. Filter 63 causes less eye strain than C4 in routine pollen counting.

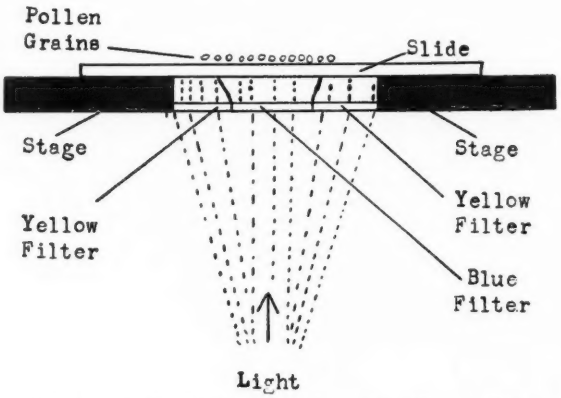

Fig. 1.—Cross section of microscope stage, color filter, pollen, and slide.

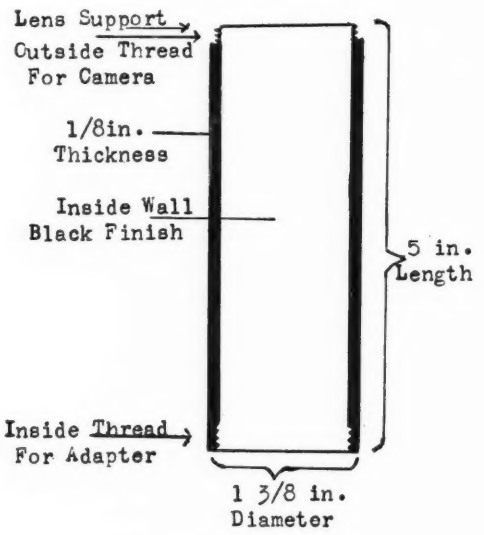

Fig. 2.—Extension tube.

If mere magnification were the answer to greater detail, then the study of pollen grains would be simple, since there is practically no limit to the extent of magnification possible. In order to avoid the

empty magnification which is associated with lack of resolution in increases in magnification, the following inexpensive addition was made to the standard microscope. Metal tubes of varying lengths were inserted in the microscope system to vary the distance of the ocular piece from the objective. After experiment it was found that a tube 5 inches long gave sharp detail (Fig. 2). Two ocular pieces were used. A

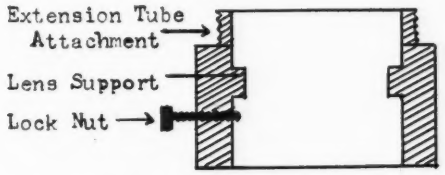

Fig. 3.—Adapter. Extension tube to microscope.

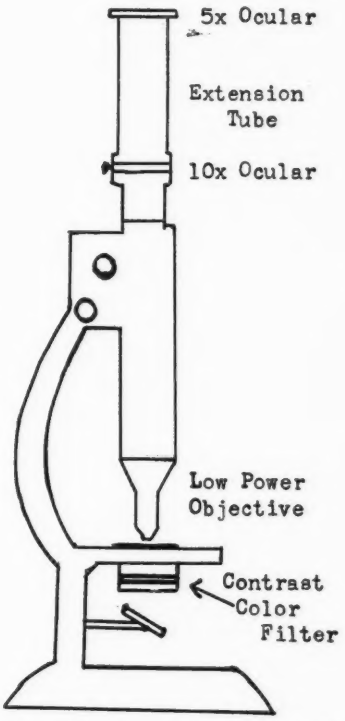

Fig. 4.—Complete assembly of lenses and filters.

5× ocular piece was placed in the upper end of the tube, and a 10× ocular piece was placed in the usual position in the microscope (Fig. 3). A low-power objective was used. This gave a magnification of 750 diameters. The magnification was computed according to the factors in the following formula:

10 (low-power objective) × 10 (10× ocular) = 100 diameters
100 diameters + 50 (5-inch extension tube) = 150 diameters
150 diameters × 5 (5× ocular) = 750 diameters

This magnification of 750 diameters, giving good detail, may be compared with the usual low-power magnification of 100 diameters which is too small and with the usual high-power magnification of 450 diameters which is too blurred for easy examination (Fig. 4).

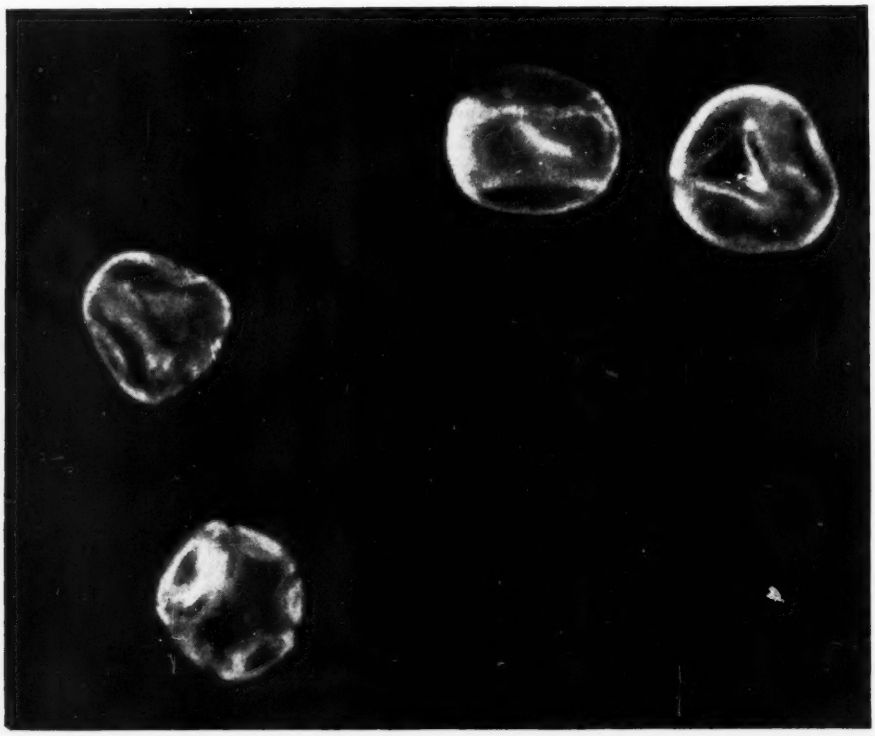

Fig. 5.—Unexpanded pollen of timothy grass. (Pollen appears brightly colored yellow on contrasting blue background.)

In order to take photographs in color, an inexpensive camera which contained a reflex ground glass mirror was used. The lens of the camera was removed, and the upper end of the metal extension tube was attached in the camera lens opening. With this camera the microscope field could be readily viewed and sharp focus obtained. After considerable experiment with various light sources and exposure times, an exposure of eight seconds with a mercury vapor lamp produced the best color photographs (Fig. 5). The lamp was especially designed for photomicrographic work and had a rating of 375 candle power. The positive color films may be seen enlarged to about 3,600 times when projected on a screen.

For making quantitative pollen counts by this method, the microscopic field may be easily measured by inserting a hemocytometer counting chamber under the objective piece. The Nebauer ruling on the counting chamber contains 400 squares per square millimeter. In each of the microscopic fields seen with the magnification used in this

problem there are about 120 squares. With this as a basis the counting may be done by any of the various computations which may be preferred.

It is hoped that this report will serve to stimulate further interest in improving methods of the examination of pollen grains. It is not unlikely that by the use of other extension tubes of varying lengths, by the use of other lenses, and by the introduction of other color contrast combinations further improvements may be made.

Mr. Rolland Jenkins, a member of the New York Microscopical Society, offered valuable suggestions on certain technical aspects of this work.

SUMMARY

1. This paper presents a microscopic method of examining pollen grains in their natural, unexpanded state.

2. Detail through color is secured by the use of color contrast filters without staining agents of any kind.

3. A magnification of 750 diameters was obtained by the use of a 5-inch extension tube, a 10× ocular piece, a 5× ocular piece, and a low-power objective in the same microscope system.

Equipment.—Standard microscope with low-power objective, 10× ocular, and 5× ocular, mercury vapor lamp—of 350 candlepower (for photography), ordinary light source (for routine examination), Rheinberg differential color illumination disks, transformer for mercury vapor lamp, extension tubes, adapter ring, and camera with reflex ground-glass mirror.

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DISCUSSION

Dr. Henry B. Wightman, New Rochelle, N. Y.—To anyone who has wrestled with the problems of pollen photography and color photography in particular, the results which Dr. Alford has presented are very gratifying.

As I see it, there are a great many advantages in this method. 1. The slides may be examined repeatedly. It is often interesting to reexamine the slide later. 2. The color does not fade as in a stained specimen. 3. The pollen is seen as it exists in the air. 4. Most important to date, the procedure is simple and inexpensive. 5. One of the most important contributions Dr. Alford has given us this afternoon

is his ingenious method of ocular lenses, whereby the pollen grain is enlarged without disturbing the depth of the focus. The bottom of the grain is not in focus and the top out, or the middle, but the whole grain has been retained in focus. That is of primary importance from the photography standpoint because the photographic lens picks up things in one plane only.

The disadvantages are of minor importance. If the grain is tremendously distorted, as in dry spells, it becomes crenated; the furrows are nature's way of preserving moisture. If the climate is dry, the furrows will become depressed, somewhat distorted, and not recognizable. In other words, there's a certain advantage to expanding all grains to their maximum size the way Dr. Wodehouse does because they will appear the same.

DR. J. A. CLARKE, JR., Philadelphia.—We have been doing differential atmospheric pollen counts for a few years. We have studied the pollen in the moist or expanded state, and I have followed the method of identification in the shrunken state with a great deal of interest. I am not convinced that it is an improvement. In the expanded state we have only one difficulty, and that is to distinguish between plantain and grass pollen. It is almost impossible at times to tell which is plantain and which is grass. In using this new method, it strikes me that one great difficulty would be to have the pollen properly dried. On the other hand, it is very easy to expand all of the pollen simply by putting it in an aqueous media, such as the glycerin jelly. I wonder if Dr. Alford has made any provision for drying his atmospheric pollen, because there are many damp days during the pollen season when the pollen, as it collects on the slide, is already swollen to its full capacity.

DR. NATHAN SCHAFFER, East Orange, N. J.—I have been doing some photography of pollen and fungi. I get very clear detail by using glycerin, sprinkling the pollen on the glycerin, and then exposing it to iodine fumes. There is no change in contour and all characteristics of the pollen may be observed.

HENOCH'S PURPURA BASED ON FOOD ALLERGY

A Report of Two Cases

Stanley F. Hampton, M.D.
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THE association of purpura of the skin with visceral and joint symptoms has been recognized for many years. Henoch[1] first described this association in 1874, and Osler[2] discussed the syndrome in 1895. In 1914, Osler[3] re-emphasized the fact that exudative skin lesions, including urticaria, angioneurotic edema, and purpura were frequently accompanied by visceral manifestations and inferred at that time that anaphylaxis might be the phenomenon responsible for the clinical syndrome. Frank[4] also attributed this group of symptoms to anaphylaxis. Duke[5] and Landsberger[6] reported cases of purpura due to food allergy. Alexander and Eyermann[7, 8] in two publications reported six cases of the Henoch's variety of purpura due to food allergy, and later Eyermann[9] reported eighteen cases of purpura. Five of the six case histories presented revealed abdominal symptoms. The two blood platelet count reports were within the normal limits. Kahn,[10] Barthelme,[11] Brown,[12] and Squier and Madison[13] have also written of Henoch's purpura attributed to food allergy.

Thomas and Forsythe,[14] in reviewing sixty-eight cases of purpura, recently reported ten cases in patients who had allergic constitutions or suffered from known allergic diseases (hay fever, asthma, vasomotor rhinitis, urticaria). Three of these cases presented gastrointestinal symptoms. Their therapeutic results were evaluated on elimination of foods and inhalants showing positive skin reactions. Two of the cases showing abnormally low platelet counts were not improved on allergic management. In no instance was the purpuric syndrome reinduced.

The following two cases of purpura of the Henoch type are presented as allergic manifestations.

Case 1.—A white female, aged 15 years, was admitted to Barnes Hospital on Aug. 28, 1940, with a history of generalized abdominal, cramplike pain, nausea and vomiting, diarrhea of six to eight stools daily, and vague joint pains for eight years. The symptoms had been more or less constant, with variation in intensity, from time to time, the longest period of complete freedom of symptoms being one week. During the year prior to admission, there had been daily purpuric spots of the skin and gross red blood in stools. An appendectomy had been performed three years previously because of the abdominal pain, without relief, and a tonsillectomy and adenoidectomy had been done six years previously because of frequent sore throats. There had

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been frequent nosebleeds, and on one occasion, when a tooth was extracted, there was profuse bleeding. The patient had observed that tomato, sweet corn, green peas, navy and Lima beans, chocolate, mustard, and black pepper increased the severity of all of the symptoms. The personal past history was negative for allergy. The family history was also negative for allergy, except for the fact that the patient's mother had suffered from a clinical syndrome similar to that of the patient but less severe. Her allergy apparently was due to the ingestion of eggs, melons, and onions.

Fig. 1.—Case 1. Roentgenogram of colon after barium enema.

A physical examination revealed a well-developed, well-nourished white girl of 15 years, with pallor of skin. There were numerous purpuric spots on the arms and legs up to the size of a half dollar. There were a few scattered petechiae on the fingers. The pupils reacted to light and accommodation, and the ocular fundi were

normal. The ears and nose were negative to external examination. The teeth and gums were within normal limits. There were small tonsillar remnants. The heart and lungs were normal. The blood pressure was 122/78. The abdominal examination revealed tenderness along the course of the colon. The spleen was palpable two fingerbreadths below the left costal margin, and the liver edge was felt at the level of the right costal border. The reflexes were physiologic. Pelvic and rectal examinations were within normal limits. Sigmoidoscopic examination revealed an edematous-appearing mucosa, with no ulceration, but many small petechiae. The mucosa bled on very slight trauma, such as that caused by touching it with a cotton swab applicator.

Laboratory Findings.—Intradermal skin tests with common inhalant and food allergens were negative. The blood Kahn was negative on two occasions. Frequent urinalyses failed to reveal any abnormal findings. An analysis of the fasting gastric secretion revealed 30 per cent of free HCl. Capillary fragility tests demonstrated two petechiae at 45 mm. of mercury negative pressure for one minute. Repeated stool examinations showed gross red blood or were strongly positive for occult blood (guaiac test). A tonsillar culture on blood agar plates showed *H. influenzae*, pneumococcus, and *M. catarrhalis*. Examination of the stools for amoebae and parasites was negative. Repeated stool cultures failed to show organisms of the typhoid-dysentery groups, but on blood agar plates showed *B. coli*, diphtheroid bacilli, *M. tetragenous*, nonhemolytic streptococci, and *B. alkaligenes*. Stool cultures on Sabouraud's media showed yeastlike organisms.

TABLE I

CYTOLOGIC EXAMINATION OF PERIPHERAL BLOOD

(Case 1)

TYPE OF CELLS	DATE							
	8/24/40	8/29/40	9/3/40	9/10/40	10/30/40	11/15/40	12/13/40	4/5/41
Red cells (per cu. mm.)	4.27M	3.77M	4.45M	4.41M	3.36M	3.86M	4.5M	4.1M
Hemoglobin (%)	63	57	79	70	61	71	91	71
White cells (per cu. mm.)	5,450	4,300	4,650	7,150	5,500	4,050	5,700	7,700
Platelets (per cu. mm.)	310,000	273,000	356,000	441,000	174,000	150,000		455,000
Differential count (% of cells per cu. mm.)								
Neutrophiles	62	51	58	55	62	63	74	44
Lymphocytes	9	27	14	23	20	16	22	17
Band	13	4	10	2	6	9	0	21
Eosinophiles	5	11	14	13	2	5	4	1
Basophiles	1	0	0	0	0	1	0	0
Monocytes	10	7	4	7	8	5	0	14
Reticulocytes		5.0	4.4	2.0		4.5		

M = Million.

Flouroscopic and x-ray examination of the stomach after a barium meal showed a normal stomach and duodenum, with normal gastric mobility. X-ray of the colon after a barium enema, however, showed a general suppression of haustrations and

some shortening of the colon (Fig. 1). Roentgenograms of the gall bladder after oral dye failed to visualize the gall bladder.

Repeated cytologic examinations (twelve in all) of the peripheral blood revealed secondary anemia, an increase in eosinophiles up to 14 per cent, and an increase in monocytes. No pathologic cells were demonstrated. Platelet counts varied between 151,000 and 441,000 which were normal by the method used (Table I). The bleeding times by the ear puncture method were seventeen and twelve minutes on two occasions. The coagulation time was normal, as was the prothrombin time. Examination of the bone marrow by means of sternal puncture showed normal marrow elements, except for an increase in immature and mature eosinophiles (Table II).

TABLE II

CYTOLOGIC EXAMINATION OF BONE MARROW

(Percentage of Cells)

(Case 1)

TYPE OF CELLS	DATE	
	8/25/40	4/5/41
Eosinophiles	8	8
Basophiles	0	1
Lymphocytes	14.5	7
Monocytes	0	0
Neutrophiles	4	6
Band	10	40
Myelocytes	50 (10% eosinophiles)	20 (6% eosinophiles)
Metamyelocytes	11	16
Clasmatocytes	1.5	0
Plasma cells	0.5	1
Megakaryocytes	0.5	1
Normoblasts	57	10
Erythroblasts	19	29

Course.—The patient was allowed no food by mouth and was given synthetic tube feedings for four weeks. The feedings, formulated by Dr. William Olmsted of the Washington University Medical School, were composed of amino acids, glucose, corn oil and gelatin, salt, and synthetic vitamins, supplying 1,400 calories. The details of this procedure are forthcoming in a separate publication by Olmsted.[15] It suffices to say here that it was assumed that such feedings would be nonantigenic, and later investigation has shown that the material is nonantigenic in guinea pigs. After three weeks of tube feedings, the pain and diarrhea ceased and the stools became negative for occult blood. The patient was discharged from the hospital. She was then fed nothing but whole cow's milk, totaling 2,800 c.c. in forty-eight hours. At the end of this period, generalized abdominal pain, joint pain, purpura of the arms and legs, and watery diarrhea ensued. The milk diet was continued for one week, and the symptoms, including gross red bloody stools, continued. The milk diet was then discontinued, and the patient was placed on a diet of nothing but wheat, salt, and sugar for a period of one week. The symptoms completely subsided forty-eight hours after the milk diet was discontinued. Beef was then added to the wheat diet for one week with no ill effects. At this time (eighteen days after cessation of symptoms) a sigmoidoscopic examination was made again, and the mucosa appeared normal, with no petechiae or bleeding sites. Stool specimens taken at this time did not contain gross or occult blood. Subsequently, eggs were added to the diet with no ill effects. Later, white potatoes were added to the diet, and after seventy-two hours the typical syndrome with bloody diarrhea, purpura, and abdominal pain occurred. The symptoms subsided within two days after white potatoes were eliminated.

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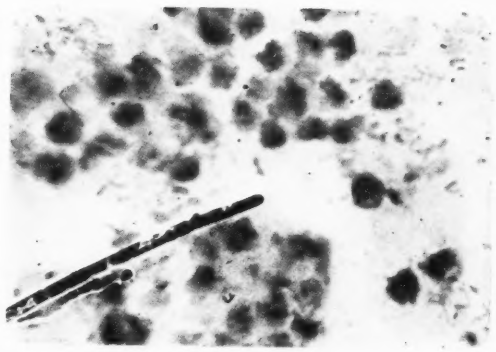


Fig. 2.—Case 1. Slide of mucus in stool showing eosinophiles.

The patient remained symptom free except for two occasions when she broke her diet by drinking milk and eating milk chocolate candy. On these occasions the symptoms lasted for twenty-four to forty-eight hours. The abdominal pain with these attacks was completely relieved by a subcutaneous injection of 0.3 to 0.5 c.c. of adrenalin hydrochloride 1:1,000.

After the diet of wheat, eggs, and beef had been established over a period of six weeks, the patient failed to return to the clinic for another six weeks. During this time she had taken upon herself to add a few foods including pineapple juice, spinach, carrots, pears, peaches, and tea to her diet, with no ill effects. At this time, however, thinking that she was cured of her disease, as she later stated, she voluntarily began to eat milk and milk products. Within four days, the complete clinical syndrome recurred. For some unknown reason she continued to take milk, and at the end of two weeks was having as many as forty bloody diarrheal stools within twenty-four hours. She was admitted to the hospital and once again obtained relief with the use of synthetic feedings. During the period of dietary limitation, synthetic vitamins in large doses were administered.

Smears of the mucus from the stools during episodes of diarrhea have shown numerous eosinophiles as well as pus cells. The staining materials included eosin with Wright's counter stain, eosin with methylene blue counter stain, Giemsa stain, and vital staining. Fig. 2 is a photograph of one of these smears.

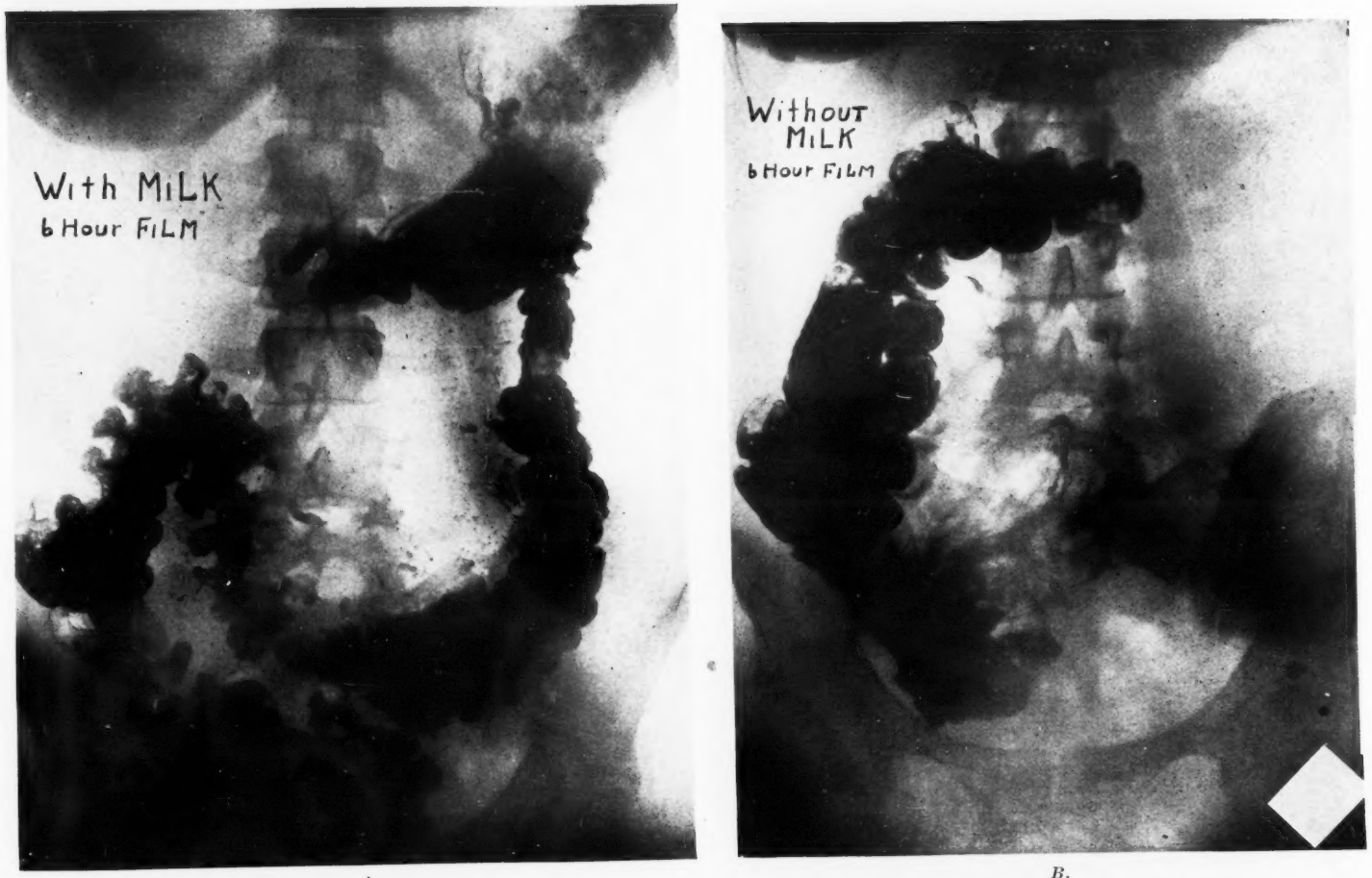
CASE 2.—A white female student nurse at Barnes Hospital, aged 20 years, had had frequent attacks of abdominal cramplike pain, nausea and vomiting, diarrhea, and painful, stiff joints, including the shoulders, knees, and ankles, for four years. The attacks occurred every few days and lasted for one to five days, with residual abdominal "soreness." The patient had noticed "black and blue" spots on the skin for several years, and some of them were associated with the attacks of diarrhea. For five days prior to the patient's admission the above described syndrome associated with definite purpuric areas of the skin had persisted. The stools had numbered up to twenty-four in twenty-four hours but did not contain gross red blood. There had never been gross red blood in the stools and no tarry stools. The attacks increased in frequency. In November, 1940, a diagnosis of recurring acute appendicitis was made, and an appendectomy was performed, without relief of symptoms.

For eight years, the patient had had perennial attacks of nasal obstruction and watery discharge, but very little sneezing. Associated with the nasal symptoms had been frequent generalized headaches. She had thought that chocolate, fish, and corn induced the nasal symptoms.

One brother has hay fever; one sister has hives; and the patient's mother has always had eczema.

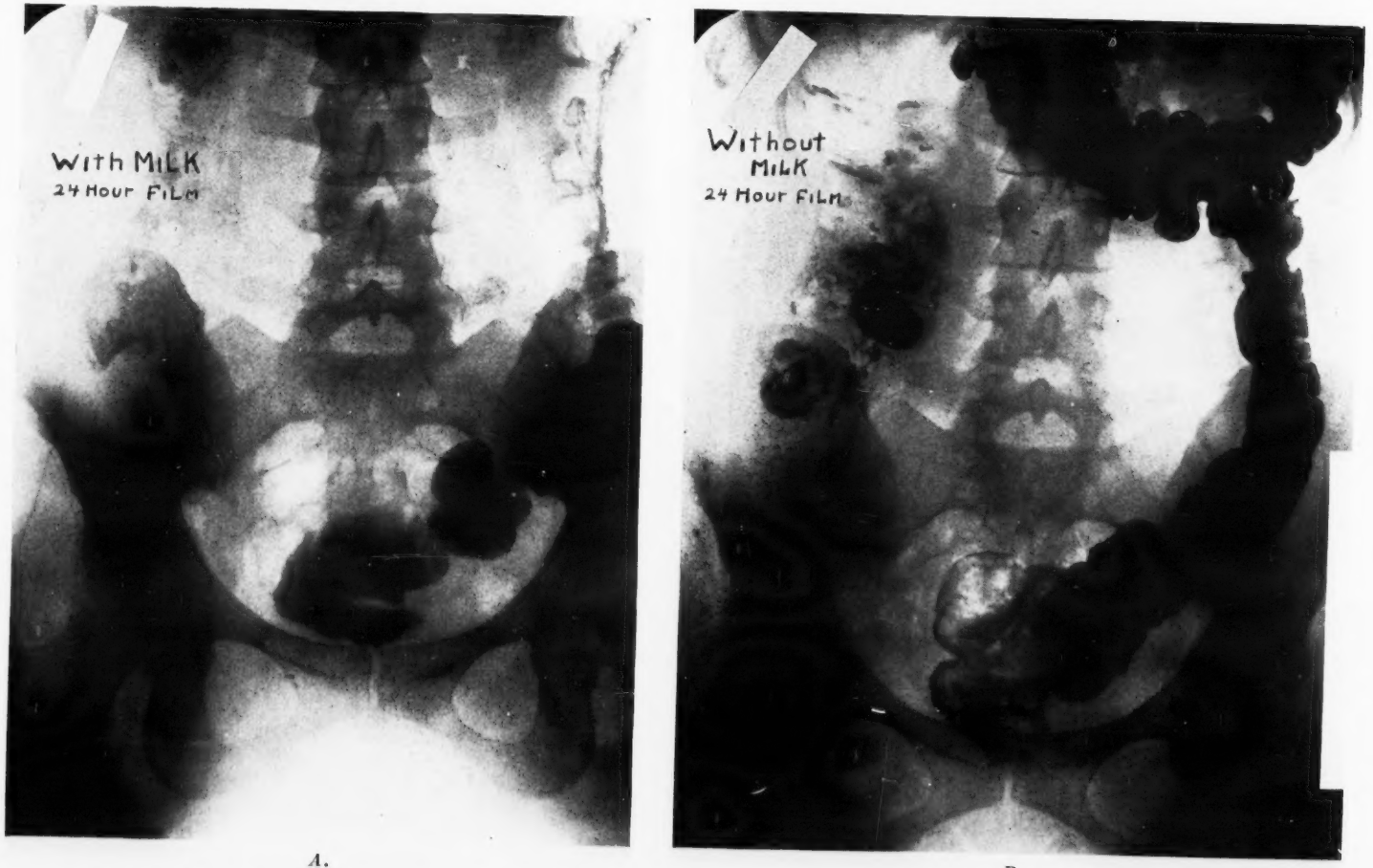
A physical examination revealed a well-developed, well-nourished white female of 20 years. There were several purpuric areas up to 3 cm. in diameter on the upper and lower extremities. No petechiae were seen. The pupils reacted to light and accommodation. The ears were normal. The nasal mucosa was of the vasomotor type. No polyps were seen. The teeth were in good repair. The tonsils were out. The thyroid was palpable. The lungs were clear. There was a faint systolic murmur at the apex and base, and a sinus arrhythmia. The abdomen presented a low right rectus scar. There was diffuse abdominal tenderness, but no rigidity. The spleen and liver were not palpable. The reflexes were physiologic. There was pain on motion of arms and legs, and the shoulders and knees were tender, but not hot or red.

Laboratory Findings.—Intradermal skin tests with common inhalant and food allergens were negative. The blood Kahn was negative. Frequent urinalyses were negative for albumin, sugar, and blood.



A. *B.*

Fig. 3.—Case 2. Six-hour roentgenograms of gastrointestinal tract with (*A*) and without (*B*) ingestion of milk.



A. *B.*

Fig. 4.—Case 2. Twenty-four-hour roentgenograms of gastrointestinal tract with (*A*) and without (*B*) ingestion of milk.

The red blood cell, white blood cell, and differential counts were within normal limits (except for an eosinophilia up to 6 per cent), as were the hemoglobin and reticulocyte determinations of the peripheral blood. The platelet count during the observed attack described above was 800,000 per cubic millimeter. The bleeding time was 2.5 minutes; and the blood clotting time was eighteen minutes (normal); and the prothrombin time was normal. The red blood cell fragility tests showed normal results. The whole blood vitamin C determination was 1.8 mg. per cent (Table III).

Fig. 5A.—Case 2. Roentgenogram of colon (barium enema) with ingestion of milk.

The stools were examined for amoebae and parasites, but none were found. Several stool examinations were negative for occult blood. A stool culture for the typhoid-dysentery group of organisms was negative, and a culture on blood agar plates showed only *B. coli*.

X-ray examination of the sinuses showed them to be clear. Roentgenograms of the gastrointestinal tract showed gastric retention and hypermotility of the entire tract with evidence of spasticity of the colon (Figs. 3, 4, and 5).

Fig. 5*B*.—Case 2. Roentgenogram of colon (barium enema) without ingestion of milk.

Course.—When the patient was first seen, she was doubled up with abdominal pain. A hypodermic injection of 0.3 c.c. of adrenalin hydrochloride 1:1,000 brought about complete relief of the pain within fifteen minutes, and the diarrhea ceased within a few hours. The pain and diarrhea recurred the next day, and complete relief was again obtained with adrenalin.

It was felt that the patient was a candidate for synthetic feedings, but she was reluctant to take them. It was decided to formulate a trial diet for a few days and, if the symptoms recurred, to resort to synthetic tube feedings. She was given a diet of lamb, chicken, pears, peaches, sweet potatoes, beets, lettuce, with olive

oil and lemon juice dressing, tea, and synthetic vitamins. The entire clinical syndrome subsided for a period of ten days. At this time carrots were added to the diet, and within three hours abdominal and joint pain, skin purpura, and diarrhea developed. Relief was obtained with adrenalin, and the carrots were removed from the diet. The patient remained free of symptoms for another ten days. At this time she was given one pint of whole milk, and the usual symptoms developed within two hours, lasting twenty-four hours. After four or five days, wheat in the form of puffed wheat was fed to the patient, and again the symptoms recurred. Beef was added to the diet with no ill effects. A few days later milk was again tried in the diet with repetition of the clinical syndrome after two hours. Wheat was again tried with the same results. The patient was discharged from the hospital upon the maintenance diet. She remained free from symptoms, and three weeks later additions to her diet were resumed. Additions were made to her diet weekly, the diet being supplied by the department of dietetics of Barnes Hospital.

TABLE III

EXAMINATION OF PERIPHERAL BLOOD

(Case 2)

W.B.C.	8,300 per cu. mm.
R.B.C.	4.84 million per cu. mm.
Hemoglobin	14.6 Gm. (94%)
Reticulocytes	2.4%
Platelets	800,000 per cu. mm.
Bleeding time	2.5 minutes
Coagulation time	18 minutes (normal)
Prothrombin time	35 seconds (normal)
Differential count (per cent of cells per cu. mm.)	
Eosinophiles	4
Basophiles	0
Lymphocytes	23
Neutrophiles	62
Myelocytes	0
Metamyelocytes	0
Band cells	4
Monocytes	7

The following foods were added with no ill effects: asparagus, banana, white potato, tomato, Brussels sprouts, and coca-cola. The following additional foods induced the clinical syndrome: apple, string beans, orange, pineapple, and prune. Each time the symptoms developed within two to four hours and lasted until relieved by adrenalin. The patient received such dramatic relief from her pain and diarrhea with adrenalin that she kept a syringe and a vial of it in her room and administered it to herself when attacks occurred.

On April 18, 1941, milk was given and roentgenograms of the gastrointestinal tract were taken. The following was recorded: 7 A.M., 1,000 c.c. whole milk fed; 9 A.M., abdominal pain, nausea, headache, nasal obstruction; 9 A.M., 500 c.c. whole milk with barium sulfate, x-ray examination; 10 A.M., diarrhea started; 2 P.M. (following day), purpura of legs, arms, and trunk.

The patient passed twenty-five stools within twenty-four hours, and adrenalin was taken four times before complete relief of the pain was obtained. The pain was excruciating. The entire syndrome, except the purpura, subsided within thirty-six hours, and she was then able to return to nursing duty.

The nasal symptoms and headaches subsided on the maintenance diet and recurred coincidentally with each induced attack of pain and diarrhea.

During the time in the hospital before symptoms were relieved, the temperature varied irregularly, reaching a maximum of 38.6° C. (101.5° F.), but remained normal after the condition was controlled.

Roentgenograms of the gastrointestinal tract were made at a time when the patient was free from symptoms and also during an attack induced by ingestion of milk. Figs. 3, 4, and 5 show the contrast of roentgenograms made after the ingestion of barium and milk and barium alone. Fig. 3 shows six-hour roentgenograms made after barium and milk and barium meals, demonstrating gastric retention with ingestion of milk. Fig. 4 shows twenty-four-hour roentgenograms made after the same meals and demonstrates hypermotility with ingestion of milk. Fig. 5 shows the colon after a barium enema during an attack induced by the feeding of milk and also when free from symptoms. There is increased spasticity under the influence of ingestion of milk.

The roentgenograms of the gastrointestinal tract are similar to those of gastrointestinal allergy published by Eyermann[16] and to those of gastrointestinal allergy to split products (proteoses) of milk demonstrated by Cooke and Hampton.[17]

SUMMARY

1. Two cases of purpura associated with gastrointestinal and joint symptoms have been described in detail. The symptom syndrome in these cases has been induced at will by the feeding of certain foods.

2. Roentgenograms of the entire gastrointestinal tract of a patient with Henoch's purpura, taken when the patient was symptom free and also during an attack of Henoch's purpura induced by the feeding of milk, show gastric retention, hypermotility, and spasm of the colon as a result of the ingestion of milk.

3. In cases of purpura of the Henoch's type, when drugs and possibly bacterial allergy can be eliminated as etiological factors, food allergy should be considered.

4. The use of nonantigenic foodstuffs, such as amino acids, administered by tube feedings, offers a method of removing offending allergens and of maintaining proper nutrition at the same time.

The author wishes to express his appreciation to Dr. Carl Moore and his laboratory staff for the hematological studies presented and to Dr. W. H. Olmsted for the preparation and application of the synthetic feedings used.

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DISCUSSION

DR. R. A. KERN.—Dr. Hampton has told us of two cases of Henoch's purpura due to food allergy with negative food skin tests and has discussed their treatment. I am reminded to tell you of a patient who had Henoch's purpura due to food sensitivity with a positive skin test of a very unusual type. This man had the classical manifestations of Henoch's purpura with the skin and the abdominal phenomena and, in addition, as his major threat, renal manifestations with hematuria and nitrogen retention, so that he arrived at the hospital with an urea nitrogen of well over 100 mg. per cent. His symptoms, we suspected, were due to certain foods, and by elimination we proved that one food alone was responsible, and that was onion. We tested the patient repeatedly and by all possible methods with onion extract, but always with negative results, until he said, "Doctor, why don't you look at these tests the next day or two?" Then we discovered that an intracutaneous test was negative for six hours, and then it began to show local swelling which was quite marked after twenty-four hours. I wonder if there did not occur at the site of the injection in the skin a partial digestion of the material, resulting in some split product which then gave a positive skin reaction. Unfortunately this patient obtained a job as an orderly when he was dismissed. On his first night his supper consisted of onion stew. He died of uremia a few days later.

DR. FRANCIS RACKEMANN.—The skin test was negative. Do we not see many patients who are sensitive in the clinical sense, but whose skin test with the specific substance is negative? I believe there is an explanation for this lack of correlation which is always so troublesome. In this patient the disease was of his bone marrow, particularly of the blood platelets. Symptoms were referable to his blood-forming organs as well as to his gastrointestinal tract. Other patients with allergy have symptoms in the respiratory tract; they have hay fever or asthma, just as a smaller group have symptoms in the skin.

Is it not true that allergy can affect certain different tissues at different times or at least one tissue more than the others? Ought we not lay more stress on the reaction of local tissues which may or may not include the skin and less stress on reactions of the body as a whole, including serologic reaction?

DR. HAMPTON.—In one of Osler's[2] first reports he reported a case of glomerular nephritis associated with or as a sequela of Henoch's purpura. Alexander and Eyermann[7] also reported such a case in 1927, which I believe presented terminal nephritis as a result of long-standing Henoch's purpura. In regard to Dr. Rackemann's remarks, I did not understand whether he was talking about his case or one of my cases. In the case presented today, the bone marrow was normal except for the increase in mature and immature eosinophilic cells. In this discussion I

did not attempt to explain the difference in histopathology of allergic purpura and thrombocytopenic purpura. It is a subject within itself. Apparently, in allergic purpura the bone marrow is normal except for an increase in eosinophilic cells.

Dr. Vaughan is right. The feeding mixture has an unpleasant flavor. One patient did attempt to take it by mouth, but it had to be discontinued after two days because of the most disagreeable taste.

1:500 EPINEPHRINE IN GELATIN

A Discussion of Its Action, Advantages, and Disadvantages

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IN THE March, 1939, issue of The Journal of Allergy, Spain, Strauss, and Fuchs reported the symptomatic treatment of chronic asthmatic patients with a mixture of 1:500 epinephrine in gelatin. Great interest has been shown in the slowly absorbed epinephrine mixture.

Keeney was the first to use a preparation of this kind for the treatment of intractable asthma. The preparation consisted of a 1:500 mixture of epinephrine in purified peanut oil. The rationale of its use was based on the fact that oils, when given parenterally, are slowly absorbed. Therefore, if epinephrine could be mixed or suspended in oil, it in turn would be slowly absorbed. Larger doses of epinephrine could then be used.

The above facts have been amply demonstrated because today many preparations are manufactured and put up in oil. Peanuts and peanut oil are well-known antigens. The author of this article has seen some very severe generalized reactions which resulted from the intramuscular use of epinephrine in purified peanut oil. In all fairness, however, it must be stated that the reactions occurred in persons who later were proved to be sensitive to peanuts. One report comes from a physician who self-administered 1 c.c. of epinephrine in peanut oil. He had an immediate generalized reaction which was relieved only by repeated injections of a 1:1,000 solution of adrenalin chloride. Peculiarly, three days after his injection of epinephrine in peanut oil, he developed urticaria, which persisted for about eleven days.

Epinephrine in oil was formerly marketed in multiple dose packages, making it necessary to give it with a dry syringe and needle. The purpose of this was to prevent contamination and subsequent oxidation of the epinephrine by water introduced from a syringe which had been sterilized by boiling. The epinephrine in oil is now marketed in 1 c.c. ampoules. It is therefore no longer necessary to use a dry syringe. The administration of the ampoules is relatively easy and requires no special technique.

Since peanut oil is an antigen and may produce toxic effects in some persons, the search for some material which would retard absorption and

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at the same time be nontoxic and nonantigenic was continued. Such a substance has been found. Gelatin is nontoxic and nonantigenic and does retard the absorption of epinephrine.

METHOD OF PREPARATION

To a solution containing 40 c.c. of glycerin and 130 c.c. of distilled water are added 1.8 Gm. of sodium chloride C.P., 1.0 Gm. of chlorbutanol, 0.2 Gm. of sodium bisulfite, and 40 Gm. of gelatin. The gelatin is added last, and the preparation is mixed vigorously by rotation. This quantity of gelatin is conveniently made in a 1 liter pyrex Florence flask since the mixture tends to boil over if it is sterilized in too small a container. The gelatin preparation is sterilized by autclaving for thirty minutes at 20 pounds pressure. Aerobic and anaerobic cultures are made and kept at 37° C. for four days before the sterility of the mixture is decided upon or the gelatin mixture dispensed for use. This gelatin mixture will remain stable for months if kept in the refrigerator. The 1:500 epinephrine solution is made by adding one part of 1:100 epinephrine solution to four parts of the sterile, gelatin mixture. The gelatin mixture must be heated in order to liquefy the gelatin so that it may be pipetted accurately. Aseptic precautions must be followed when the epinephrine is added and in the subsequent method for bottling. This 1:500 epinephrine in gelatin solution is dispensed in sterile, brown glass vials.

The mixture congeals quickly upon cooling. This is a distinct handicap because the material must be liquefied before it can be administered. If some method could be found which would keep the epinephrine in gelatin mixture in a liquid state, and at the same time preserve the slow absorptive quality of the product, one would have, in my opinion, an ideal solution for the relief of intractable asthma.

METHOD OF ADMINISTRATION

A bottle of epinephrine in gelatin mixture is heated in a water bath at about 30° C. until liquefied. The required amount of the liquefied solution is then drawn into a preheated syringe. Due to the fact that the epinephrine in gelatin mixture is put up in bulk, careful aseptic precautions must be observed, because gelatin is an excellent culture medium and can very easily be contaminated. The author has had several vials of epinephrine in gelatin mixture brought into the hospital for inspection from outside sources with profuse growths of mold present on the top and in the gelatin.

When epinephrine in gelatin mixture was first introduced in our various ward services, I advocated the subcutaneous method of administration to all patients. Blanching would appear at the site of injection in from 2.5 to six minutes. Practically all of the patients complained of a burning and smarting sensation. Several interns on

the ward service also noticed that, when the preparation was given subcutaneously, a nodule would appear at the site of the injection. These nodules would persist for three to twenty-two days. At times, a sterile and painful localized reaction also occurred around the nodule and persisted for an average of three days. Later the nurses and interns were instructed to give the preparation intramuscularly. No nodules or local reactions were noticed after the adoption of the intramuscular administration. Local blanching usually appeared at the site of the injection in about six minutes.

METHOD OF DETERMINING BLOOD SUGAR

Blood sugar responses to epinephrine hydrochloride solution 1:1,000 and to epinephrine in gelatin were determined in each patient after a twelve- to fourteen-hour fasting period. During this time, the patient rested quietly in bed and was allowed no visitors, alcohol, food, or water. At the end of this period a specimen of blood was taken, and the blood sugar was determined. The patient was then given 1 c.c. of epinephrine in gelatin intramuscularly. Specimens of blood were taken every thirty minutes for two hours and then at longer intervals for twelve to fourteen hours after the injection.

It was found that, when the 1:1,000 solution of epinephrine was used, the highest blood sugar level was generally reached in about forty-five to seventy-five minutes. After this, a fairly rapid fall took place over a period of three to 4.5 hours, when it usually dropped to below the original point.

When the epinephrine in gelatin was given, the blood sugar reached its peak in about 2.5 to three hours and persisted at a slightly lower level for seven to nine hours.

The effect of epinephrine on metabolism is interesting. It is a well-known fact that the glycogen is mobilized in the liver. Ephedrine causes its conversion into glucose, and there is a temporary increase in blood sugar. The slowly absorbed epinephrine in gelatin prolongs this effect. Because of the above-mentioned facts, it is now our custom to give our patients several drams of lactose in fruit juice shortly after they receive their injection of 1:500 epinephrine in gelatin. The ingestion of a rapidly absorbed sugar seems to give the patients a greater sense of well-being and in turn helps to supply sugar and, therefore, energy when it is needed. As an incidental finding, it has also been noticed that the patients who received the sugar obtained relief more quickly and also were less likely to have a recurrence of their attacks.

EFFECT ON PULSE AND BLOOD PRESSURE

Careful pulse and blood pressure records were kept on all ninety-two patients studied. The appearance of nervousness and pallor was also noted. Both systolic and diastolic pressures of the patients treated rose

gradually for about three hours after the injection. Most of the pressures then remained on this level for an average of six hours and then, in most cases gradually dropped back to the original readings; in a few cases the drop was abrupt.

Two of the patients had abrupt rises in their pressures and pulse rates over a short period of time. The decline in their pressures and pulse rates also took place rapidly. The rapid rise and decline probably were due to the injection of a small amount of the 1:500 epinephrine in gelatin solution into a small vein. These two patients also experienced untoward symptoms such as nausea, vomiting, precordial pressure, severe headache, pallor, and nervousness. It is my custom always to retract the plunger of the syringe to determine if the needle may have entered a blood vessel. The above reactions probably were due to the jelling of some of the material in the end of the needle which prevented blood from flowing into the syringe when the plunger was retracted.

The pulse rates of eighty-six patients increased rather rapidly during the first three hours and then gradually dropped to the original readings over a period of about six hours, only to increase again when the effect of the epinephrine had worn off. The entire effect took place over a period of about eleven hours. Three of the patients treated had no appreciable rise in their pulse rates; these patients were accustomed to taking large doses of epinephrine. One patient used as high as 80 c.c. of 1:1,000 solution weekly. These three patients did not get as much relief from their injections as did the other patients.

A fourth patient had a gradual rise and then an abrupt fall in her pressure. She became nauseated and vomited shortly after the fall took place. This occurred about five hours after she had received 1 c.c. of 1:500 epinephrine in gelatin solution. She was given 50 c.c. of glucose intravenously, and the nausea and vomiting disappeared. The injection did relieve her asthma, and, even though she had suffered discomfort from the material given her, the patient repeatedly asked for injections because of the relief obtained.

The length of the intervals between injections varied with the patients treated. Most of those who received treatment were intractable asthmatic patients, and the usual dose was 1 c.c. of the 1:500 epinephrine in gelatin shortly before retiring. Eleven of the patients slept through the night without additional injections. Three required additional doses from seven to nine hours after they had received their first one.

An injection of 1:1,000 epinephrine hydrochloride was tried on each patient previous to the use of epinephrine in gelatin to ascertain whether or not the patient was epinephrine-fast.

In general, the action of epinephrine may be best described as "sympathomimetic" since it parallels closely the action of the sympathetic nervous system. In those structures in which the sympathetic fibers are

motor, epinephrine causes stimulation. In those to which the sympathetic fibers are inhibitors, epinephrine is likewise inhibiting. Epinephrine is an effective and powerful vasoconstrictor. This action is most marked in the arterioles because here the smooth muscle is relatively most abundant. The general effect of the vasoconstrictor is elevation of the blood pressure. The degree of the rise is dependent upon the dose of epinephrine and the rapidity of absorption of the solution or mixture given. When an aqueous solution of 1:1,000 epinephrine hydrochloride is used, the tension increases rapidly and also returns to normal a short time after the injection is given. This is due to the fact that epinephrine is oxidized quickly in the body, the change taking place largely in the blood vessels and liver.

On the other hand, the ability of the vessels to respond to epinephrine stimulation is not exhausted rapidly. The vessels will continue their constriction as long as there is epinephrine in the blood stream. The blood pressure may thus be maintained indefinitely at an elevated level by means of a slowly absorbed epinephrine mixture.

The effect of epinephrine on the heart is twofold—direct and indirect. The direct effect is one of stimulation and acceleration, with stronger contractions, and, as a result, more complete emptying of the chambers. The indirect action comes into effect as a result of the rise in pressure. The elevated pressure causes a vagal stimulation, and this in turn results in slow, full, forceful heart action, characteristic of vagal inhibition. As the blood pressure starts to fall, the direct effect again gains the upper hand, and the heartbeat again is accelerated somewhat. This effect of epinephrine on the heart explains why the pulse rate of a recipient of epinephrine in oil or gelatin first rises, declines gradually, and then again goes up for a short period of time.

The action of epinephrine on the bronchial tree is very important. It relaxes the smooth muscle in the bronchi and thus dilates them widely. It is this action that is important to the asthmatic patient. Again, a slowly absorbed epinephrine solution, suspension, or mixture is very important because the dilation of the smooth muscle in the bronchi can be maintained over a longer period of time, and this results in longer relief for the asthmatic patient who receives the injection.

CASE REPORTS

CASE 1.—Mrs. R. S., aged 51 years, had asthma for nineteen years and hay fever (fall variety) for twenty-four years. Attacks of asthma occurred perennially with severe exacerbations during late August and early September, at which time she would have from six to fifteen daily, necessitating a corresponding number of injections of epinephrine hydrochloride for relief.

On Sept. 3, 1940, she received 1 c.c. epinephrine in gelatin at 8:00 A.M. and again at 10:00 P.M. She had no attacks of asthma on this date although her environmental surroundings were exactly the same as on previous days. The next day she received no epinephrine in gelatin and again experienced frequent attacks. The

following day she was given an injection of epinephrine in gelatin twice daily and again had no attacks of asthma.

After these injections she also obtained prolonged relief from her hay fever symptoms for twelve to twenty-two hours.

This patient complained of nervousness and mild nausea shortly after she received her injections. Nausea was relieved by the administration of 2 drams of lactose in orange juice. Later the patient received one glass of orange juice plus 2 drams of lactose with her injections of epinephrine in gelatin and consequently had no more nausea; however, she continued to complain of nervousness.

CASE 2.—J. B., a farmer, aged 55 years, suffered from intractable asthma and was found to be violently sensitive to dust, cattle hair, horse hair, chicken, duck and goose feathers, cottonseed, and the early summer group of grasses. His entrance into the barn for a few minutes would precipitate an attack of asthma which usually would last several days. He could obtain no relief from the inhalation of 1:100 adrenalin chloride. He obtained only transitory relief following the hypodermic use of 1:1,000 solution of epinephrine hydrochloride. His physician decided to try adrenalin in peanut oil. Upon receiving one injection of this material, the patient became violently nauseated and vomited; he developed severe angioneurotic edema accompanied by extreme dyspnea, cyanosis, swelling of lips, tongue, eyelids, and uvula. He also developed a wheal and extreme swelling at site where the peanut oil mixture was given. After the prolonged administration of 1:1,000 solution of adrenalin chloride his symptoms gradually subsided. About twenty-eight hours later he developed an attack of urticaria which persisted for about six days. This patient was tested with peanut extract and was found to be violently sensitive to high dilutions of this material.

Various members of the staff decided that J. B. would make an ideal patient on whom to try epinephrine in gelatin mixture. He was given an injection of 1 c.c. at 8:00 A.M. and had no asthmatic symptoms until 5:00 P.M., at which time he received another injection. His symptoms returned at 3:00 A.M., and he was given another 1 c.c. injection. Although we tried to reduce the number of daily injections, it was necessary to give this patient three 1 c.c. doses daily until his symptoms completely disappeared. Desensitization therapy and environmental changes have since made him comfortable.

DISCUSSION

The 1:500 epinephrine in gelatin solution is an excellent addition to the drugs used in the treatment of intractable asthma and various acute, prolonged allergic conditions. One of its advantages over adrenalin in oil is that it is nontoxic and nonantigenic and may be used in persons who are sensitive to peanuts and thus to peanut oil. One of its disadvantages is that the mixture must first be liquefied and then drawn into a preheated syringe before it can be administered—in contradistinction to adrenalin in oil, which can be given easily in a syringe sterilized by boiling. A disadvantage of epinephrine in gelatin is that it is put up in multiple dose packages. It must be used carefully so that the bulk package is not contaminated. A fairly large number of patients who have taken both the oil and gelatin mixtures seem to prefer the gelatin because of the prolonged relief that they obtain following its use. I have found that thin people prefer the intramuscular injection of

adrenalin in oil to the epinephrine in gelatin mixture. The gelatin seems to produce a more painful localized reaction in thin persons. I believe that both the oil and gelatin preparations have distinct advantages.

SUMMARY

1. The method of administration of 1:500 epinephrine in gelatin is discussed.

2. The action of epinephrine in gelatin and its effect on the blood sugar, pulse rates, blood pressure, and bronchial tree are discussed.

3. The advantages and disadvantages of 1:500 epinephrine in gelatin are reported.

4. Some unusual reactions following the injection of 1:500 epinephrine in gelatin mixture are described.

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ABSTRACTS AND DISCUSSIONS OF THREE PAPERS READ AT THE ANNUAL MEETING OF THE SOCIETY FOR THE STUDY OF ASTHMA AND ALLIED CONDITIONS, ATLANTIC CITY, N. J., MAY 3, 1941

Studies on Human Hypersensitivity to the Poison Ivy Plant (Colored Motion Pictures). EDMUND L. KEENEY, M.D., Baltimore, Md. (Abstract.)

This past year the City of Baltimore established the first Municipal Poison Ivy Clinic. The primary purpose of this clinic was to find suitable methods to decrease compensation disability for poison ivy dermatitis among the city employees. Of 326 men who reported to the clinic, 142 (44 per cent) gave a positive history of poison ivy dermatitis. In this positive history group, 13 per cent gave a history of having had one or more of the common allergic diseases, and 16 per cent gave a positive family history of poison ivy dermatitis. In the negative poison ivy history group only 2 per cent gave a history of having had one or more of the allergic diseases, and only 5 per cent gave a positive family history of poison ivy dermatitis.

MOTION PICTURES

Part I.—The pictures demonstrate the attempt made to discover a substance that would block the experimental poison ivy reaction. Ferric chloride solution, potassium permanganate solution, sodium perborate in vanishing cream, vanishing cream, manganese chloride solution, extract of jewelweed, and rancid butter were used. Ferric chloride solution alone possessed the ability to block the poison ivy reaction.

Part II.—The methods employed in determining the reacting ability of the skin at different sites on the body in hypersensitive persons are demonstrated. The skin of the abdomen and of the back showed a reacting ability which was better coordinated with the clinical findings and the history (of a hypersensitive person) than that showed by the skin at other sites on the body.

Part III.—The pictures show that a positive patch test produces a refractory period in the state of hypersensitivity which persists for at least six to eight weeks. The possibility that this observation might lead to a simple method of providing protection against poison ivy dermatitis is intriguing.

Part IV.—The absolute effectiveness of a 10 per cent ointment of ferric chloride and the partial effectiveness of a 10 per cent ointment of ferrous sulfate in blocking the experimental poison ivy reaction are shown in the pictures.

From the results of these experimental studies the 10 per cent ointment of ferric chloride should protect clinically against poison ivy dermatitis if it is applied before contact with the poison ivy plant occurs.

DISCUSSION

DR. W. C. SPAIN, New York City.—I believe that Dr. Keeney has demonstrated very forcibly why we see such long lists of various therapeutic preparations, ointments, liniments, lotions, powders and other pharmaceuticals designed for poison ivy treatment. Apparently the only ones that are helpful are those that are used before exposure to poison ivy, as he has shown to be the case with ferric chloride. Of course, the blocking idea is very intriguing and, from his presentation, very helpful. Mechanic's soap, such as is supplied to workers, in black greases, oils, and stains to apply in a thin film to the arms, forearms, and hands before contact with these materials, will help a great deal in people such as Dr. Keeney mentioned who have to come in repeated occupational contact with poison ivy plants.

The question of refractoriness following patch tests is a new idea in poison ivy treatment. Certainly it did not seem possible a number of years ago when Dr. Cooke and I tried to produce a period of refractoriness in patients treated with injections of the specific oleoresin. Some dozen or so patients in 1927 were studied, the active principle of the ivy plant being administered over a period of time, a matter of a year in some instances, by weekly injection and daily oral doses. These patients were tested before, during, and after treatment. Four tests were given to each patient. One test was removed after an application interval of four hours, the next eight, the next twelve, the next twenty-four, these tests being studied to see whether there was any variation in the skin reactivity to ivy extract before, during, or after treatment. We were unable to show any difference in the reactions obtained before and after treatment.

I should like to emphasize one other point. Many of the puzzling cases of poison ivy where the mode of contact cannot be demonstrated can often be explained by discovering that there is a secondary agent, the dog usually, which conveys the excitant. Sometimes a cat, a horse, or even a cow may be the intermediary, as well as contaminated clothing which may have been hanging in the closet for some length of time.

DR. HARKAVY, New York City.—The point brought out by Dr. Keeney in regard to skin refractoriness following patch tests suggests that the skin is temporarily desensitized. This is in line with an important principle established by Besredka in respect to the development of local tissue immunity in staphylococcus infections of the skin through topical applications of staphylococcus filtrate. The local use of poison ivy extract in ointment form may apparently, therefore, also be an effective method of treatment in cases which do not yield to the usual intracutaneous injections. This idea has actually been utilized by us in the treatment of difficult cases. A few years ago, Dr. F. Maisel of my clinic published some observations dealing with a patient who was highly susceptible to poison ivy and refractory to every known method of therapy. The dermatitis in this case was finally controlled and prevented by daily baths into which poison ivy extract was introduced in increasing concentrations. In other words, local desensitization of the skin by means

of direct contact with the poison ivy extract irrespective of the vehicle, represents the counterpart of the poison ivy ointment form of therapy. This method of desensitization may be utilized not only in the treatment of poison ivy dermatitis but probably in other forms of contact dermatitis recalcitrant to the usual forms of treatment.

DR. KEENEY.—We have not used clinically the ferric chloride ointment or the solution. We are getting ready to do this in the spring. Of course, I believe that one has to be on the lookout for those features that Dr. Tuft has pointed out. For that reason I have advised patients, who are campers and who do not get poison ivy unless they come in contact with the plant, to buy some cheap cotton stockings, soak them in ferric chloride solution, let them dry, and wear them on the camping trips. City employees could treat their overalls or socks in the same manner and perhaps use the ointment about the arms and hands. These employees have only sporadic contact with the poison ivy plant. We know when they are going to have contact with the plant, and, therefore, the frequency of the applications can be fairly well controlled.

Treatment With Oral Ragweed Pollen. ELIZABETH H. ILIFF, M.D., and LESLIE N. GAY, M.D., Baltimore, Md. (Abstract.)

During the summer of 1940 eighty-two patients were treated with oral ragweed pollen (Panoral, Lilly). Sixty-two of the patients (75.6 per cent) were treated preseasonally, and twenty (24.4 per cent) coseasonally. The dose was started at 500 Noon units and increased gradually to totals varying from 60,000 to 240,000 units. The preseasonal cases were treated two or three times a week before the season, and all cases daily during the season.

Two patients with preseasonal treatment obtained practically complete relief of symptoms. Eleven (17.7 per cent) of the preseasonal patients and none of the coseasonal obtained good relief. Twenty-five (40.3 per cent) of the preseasonal and fourteen (70 per cent) coseasonal had fair relief. Thus a total of fifty-two (63.4 per cent) were somewhat benefited. Twenty-five patients (30.5 per cent) failed to obtain any benefit, and five (6.1 per cent) stopped treatment because of reactions.

Reactions occurred in thirty-three patients (40.2 per cent). General systemic reactions, all mild, were urticaria, pruritus, and flushing and burning of the face with swelling of lips (one case each). Six patients (7.3 per cent) reported very mild hay fever symptoms, and one had a suggestion of asthma after the first two doses. Twenty-five (30.5 per cent) had some degree of gastrointestinal discomfort. One patient had severe cramps, vomiting, and diarrhea and stopped treatment. Ten others (12.2 per cent) had moderate symptoms, and three of these discontinued the treatment. Fourteen (17.1 per cent) experienced mild nausea or slight cramps.

Of the twenty-five patients who failed to benefit from treatment, one had pansinusitis during the season and eleven gave definite evidence of

other allergy or of psychogenic factors. Two were treated coseasonally too late to be sure of the results. Three other patients had received previous injection treatment without benefit.

Blood reagins were determined in seven patients. Blood samples were drawn before treatment, at start and end of ragweed season, and (in three cases) also in midseason. Two of the seven failed to show any reagin in a 1:10 dilution. Another showed no change in the three specimens. Two showed an increase in the last specimen only, while in two instances there was an increase in each of the last two specimens. In no case was there any change in reagin titer between the beginning of treatment and the start of the ragweed season.

DISCUSSION

Dr. Samuel Feinberg, Chicago.—The experience of Dr. Iliff and Dr. Gay is pretty much in agreement with the experience of most of us with oral pollen therapy; that is, approximately 20 per cent obtain what we term good or excellent results, and the remainder are fair, poor, indifferent, or undecided. In 1937 we treated twenty patients, mostly coseasonally, and we felt certain that at least eighteen of them received no benefit.

In 1939, three or four university clinics in Chicago and the University of Michigan cooperated in a joint study. Each clinic treated its own patients and formed its own conclusions independently, but a definite program of procedure was formulated for all groups at the beginning of the project. At the end of the experiment the results were compared. Satisfactory results varied in 15 to 29 per cent of the patients, the lowest being in my group and that of Dr. Sheldon. The highest percentage of satisfactory results, 29 per cent, occurred in the University of Illinois clinic. This clinic also had a control series receiving placebo capsules of the same color and of the same starch content, but without pollen, and 25 per cent of that series obtained satisfactory results. In one of the other groups placebo injections of normal salt solution produced beneficial effects in some patients. It is significant that none of us obtained any results with respect to asthmatic symptoms, and we made the statement at that time that "since asthma is almost always a later manifestation in the progress of pollinosis and since in hypodermic therapy relief obtained from the asthma is more certain and apt to occur before the relief of hay fever, more reliance can be placed on asthmatic symptoms in evaluating the results."

The reactions in our clinic at the Northwestern University Medical School were almost exclusively gastrointestinal. A few patients in the other clinics were thought to have other types of reactions, such as hay fever, but these symptoms were not sufficiently striking to be conclusive. Gastrointestinal reactions occurred in 30 to 40 per cent of our various groups. In one of our 1937 series we were certain that we obtained an esophageal reaction. At that time we were using pollen extract orally, and one patient misunderstood the directions and swallowed 0.5 teaspoonful doses of the concentrated extract without diluting it. A few days later she returned, complaining that every time she took the "medicine" she could not swallow food for the next few hours.

The question of the absorption of the pollen antigen from the gastrointestinal tract is pertinent to this problem. In 1937 Dr. Bernstein and I investigated this phase in seventeen nonallergic subjects in my medical ward at the Cook County Hospital. One forearm of each subject was sensitized with dilutions of ragweed reagin, and in each case the subcutaneous injection of 15,000 pollen units of ragweed extract produced urticarial reactions in sites having serum dilutions as high as 1:64. At another time we sensitized the opposite arms with the same serum and noted the effect of oral pollen administration. In sixteen of these seventeen subjects, pollen or its extract, taken by mouth in amounts as high as 5 Gm. (5,000,000 pollen units), produced no reaction in any of the sensitized sites. In one patient with a bleeding carcinoma of the stomach reactions occurred in sites sensitized with reagin as high as 1:8 serum dilution. In other words, in sixteen of seventeen persons we could not demonstrate any evidence of sufficient absorption of the antigen to circulate, reach the site, and react.

Now, let us stop for a minute and think about these figures. In sixteen of seventeen patients, 5,000 mg. by mouth will produce no reaction, but hypodermically 50 mg. will produce a reaction in sites sensitized with serum as high as 1:64 dilution. It would take at least 64 times 5, or 320 Gm. of raw pollen, about 1.25 pounds, by mouth before one could expect to obtain a sufficient antigen concentration in the circulation comparable to what is ordinarily a maximum dose of pollen extract hypodermically. Now, if we assume further that here we are dealing with a nonallergic subject and we know that allergic subjects have more difficulty in absorbing antigens, the amount would probably have to be still greater in order to produce the proper titer of antigen in the circulation. This is beyond the realm of probabilities, especially with the difficulty of gastrointestinal reactions. It does not sound practical, unless it can be assumed and can be proved that we are dealing, as has been intimated here in one of the other papers, with two antigens, one which combines with a reagin and which gives us the skin test on the sensitized side and another which is an immunizing antigen.

As far as the so-called protective antibody is concerned, in two patients who had large amounts of pollen by mouth we were not able to demonstrate any of it in the circulation at the end of such a course of treatment. However, Dr. Cooke has suggested that there might be a third antibody. I hope there is, because it may give us grounds for the explanation of immunologic puzzles thus far unsolved.

Dr. Leslie N. Gay, Baltimore.—Dr. Feinberg's remarks might be subjected to considerable contradictory criticism, but time does not permit further digression. However, we have been interested in several of the phases of the discussion; for example, just what does happen to dry pollen or pollen extract after gastric juice has acted upon it? Our extract, which is standardized at 50 mg. per cent of total nitrogen and at 22 mg. per cent of protein nitrogen, contains 2.3 mg. per cent of amino acid nitrogen. This extract was subjected to the digestive action of normal gastric juice, and it was found that, after the lapse of two hours, the amino acid nitrogen had increased to 10.4 mg. per cent. The fate of dry pollen under similar treatment is of importance, it seems to me, as our knowledge of the amino acids suggests that they are nonantigenic.

The observations which we have made indicate that a limited number of patients derive relief from the oral administration of dry pollen.

But we do not believe that the results are so encouraging that the older hypodermic method of treatment will be supplanted. However, during the present emergency, the hypodermic treatment may be very inconvenient for the draftees; perhaps under such circumstances oral therapy might prove useful.

DR. WALZER.—There is one point about this type of treatment I do not understand. Dr. Feinberg has outlined some of the difficulties we have to face, but he did not explain how or when the capsules should be administered.

Feeding of an antigen after a meal prevents the absorption of the unaltered antigen. This was first pointed out by Brunner in 1925. We know that, if one takes the cottonseed meal (which we discussed this morning) after breakfast, there will be no absorption reaction on the arm. If one takes it after 0.5 Gm. of dilute hydrochloric acid, there will be a considerable diminution; if one takes it after an oil, such as olive oil (1 to 0.5 tablespoonful) there will be considerable diminution in the absorption of the cottonseed antigen which follows.

Now, if the people who advocate this treatment give it after meals, the absorption of antigen is decreased immediately. If this treatment is to be given a fair trial, it should be administered in the morning before meals.

DR. FEINBERG.—The seventeen nonallergic subjects to whom I made reference received pollen before breakfast in each case under standard conditions without exercise, food, or water. At least I feel that in that particular group the question which Dr. Walzer has mentioned has been eliminated. Of course, in the therapeutic trials we have usually followed what has been recommended, the post-meal feeding.

DR. M. DE M. TOUART, New York City.—I have had the benefit of long experience with this kind of treatment and have treated some patients since 1919. I have used all forms of extracts, pills, capsules, and liquid extracts and have administered them at different times of the day. About six or seven years ago I began to use the whole untreated pollen in capsules, and long before that I had decided that the only time to give this treatment properly was before breakfast, as Dr. Walzer has mentioned.

The dose to be given to a patient will vary. Some of my patients take a top dose of 1 Gm. of the pollen. Other patients have done well with 10 mg. I do not believe that there is any objection to giving the material by mouth. At first the patient can be given a daily dose, but later treatments should be spaced three or four days apart and doses should become larger; then treatment should be given once a week.

The question of asthma was mentioned. A patient under my care for seventeen years was proved to have ragweed pollen asthma. Oral pollen treatment has controlled the asthma.

DR. W. T. VAUGHAN, Richmond.—Our experience has been very much like those described. Most of our oral treatment has been given coseasonally, and about 20 per cent of the patients show good results. We do not give capsules. The patient takes the extract from a medicine dropper. Not for any scientific reason particularly but for reasons of facility, the patient is advised to take it before breakfast.

There are certain situations in which, when it does work, it is a splendid alternative method. Dr. Gay has mentioned its use in the army. One of my patients who travels has taken preseasonal oral treatment for three years with satisfactory results.

We have seen two patients who have developed fatty atrophy in the arm at the site of injections and very definite depressions in the arm after repeated injections. One of these has been given coseasonal oral treatment with good results.

Dr. Henry B. Wightman, New Rochelle, N. Y.—It is of interest with reference to Dr. Vaughan's reference to note an historical reference to oral pollen in the homeopathic literature. In 1890, in a book published by the Hahnemann Publishing Co., ambrose is suggested for the treatment of hay fever. The preparation is the tincture of fresh flower heads and young shoots. The dose given is homeopathic.

The Importance of Certain Antigenic Fractions of Ragweed Pollen in Relation to Constitutional Reactions During Treatment. William B. Sherman, M.D., and Selian Hebald, M.D., New York, N. Y. (Abstract.)

Most of the ragweed hay fever patients who received injections of pollen extract soon developed a blocking antibody for one antigen (Fraction 1) in the extract, but only a few developed blocking antibody for the second antigen (Fraction 2). If the blocking antibody was a factor in the development of tolerance for pollen extract, patients sensitive only to Fraction 1 might be expected to tolerate large doses of pollen extract better than those sensitive to both fractions.

A group of 221 ragweed hay fever patients were tested intracutaneously with the fractions. Of ninety-three who gave larger reactions to Fraction 1 than to Fraction 2, thirteen (14 per cent) had constitutional reactions during treatment. Of 108 patients who reacted equally to the two fractions, eighty-one (75 per cent) had constitutional reactions. Of twenty who gave larger reactions to Fraction 2, sixteen (80 per cent) had general reactions.

Tests with solutions of Fraction 2 gave a better idea of the probability of the occurrence of constitutional reactions during the course of treatment than did tests with ordinary extract. Of seventy-nine patients who gave negative or slight reactions to test with Fraction 2 (100 protein nitrogen units per cubic centimeter), ten (13 per cent) had general reactions during treatment. Thirty-four (53 per cent) of sixty-four patients who gave moderate reactions to test had constitutional reactions. Of seventy-one who gave marked skin reactions, sixty-two (87 per cent) had constitutional reactions. Similar results were obtained when sensitivity to the two fractions was tested by the method of passive transfer.

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It was felt that skin tests with these fractions might be helpful in planning the dosage of ragweed pollen extract for treatment of the hay fever patient.

DISCUSSION

DR. STULL.—I have followed with a great deal of interest the work reported today by Dr. Sherman and Dr. Hebald. The study of these two antigenic fractions from ragweed pollen has helped explain a number of experimental and clinical results which had been very puzzling. For example, in previous work with modified pollen extracts the heated ragweed extract in some patients produced many constitutional reactions and active skin tests, while in others it gave only slight skin reactions and did not produce much local or systemic reaction. In these cases the heated extract and Fraction 2 gave similar skin reactions. This is in accord with the experimental findings. Fraction 2 was relatively heat stable in its capacity to produce skin reactions in patients sensitive to it and in its desensitizing capacity in the Dale test. However, in both of these respects Fraction 1 was practically inactivated by heating to 70° C. The heated extract in treatment produced little or no inhibiting antibody, and Fraction 2 in most cases produced none.

In regard to the chemical nature and composition of these fractions, Fraction 1 contained 12 to 13 per cent nitrogen but no reducing sugars and was odorless and characteristic of protein. It was coagulated on heating. Fraction 2, as originally reported, contained only 1.75 per cent nitrogen and a considerable amount of reducing sugars. Recent work on precipitation and purification in a narrower range of ammonium sulfate concentration has led to further purification of Fraction 2 with a separation of Fraction 2 from reducing sugars so that at the present time Fraction 2 contains 12 per cent nitrogen, is colorless, and does not reduce Benedict's solution even after hydrolysis. In composition and chemical nature it resembles plant proteoses. It is not coagulated on heating. These different preparations of Fraction 2, when diluted on their protein nitrogen basis, have given comparable skin reactions both by direct test and by passive transfer, and they have shown comparable specificities in the Dale test, indicating that the carbohydrate was an inactive contaminant in the original preparation. The present clinical results, as well as results of animal experiments, indicate that these two fractions contain the important allergic and antigenic activity of the pollen extract. Purification of a water-insoluble, proteinlike material which precipitates at pH 4 has indicated that the material is unimportant in allergic activity as well as poorly, if at all, antigenic in animals.

Original Articles

PSYLLIUM SEED SENSITIVITY

Case Report

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PSYLLIUM seed, by virtue of its mucilaginous character, has been used as a laxative since the sixteenth century. No case of sensitization to this substance is on record so far as we have been able to ascertain. This is all the more surprising when one realizes that a member of the same plant family, English plantain, is one of the most common hay fever antigens.[1, 2]

Psyllium seed loomed into popularity in this country as a laxative and demulcent about fifteen years ago. It has been used quite extensively for the same purpose in Europe and Asia for more than a century. In addition, its mucilage has been employed in Europe as a cosmetic and in the stiffening of fabrics. The chief commercial varieties employed as a laxative and demulcent in the American trade are the French or black psyllium seed, the blonde or white psyllium seed, and the German psyllium seed.[3]

CASE REPORT

Mr. R. G., a 27-year-old employee of a pharmaceutical concern, first seen April 23, 1936, complained of symptoms of vasomotor rhinitis and gastrointestinal allergy. He dated the onset of his disturbances fourteen months prior to consultation, when he was first engaged in demonstrating a laxative containing ground psyllium seed. He attributed his symptoms to the product he was demonstrating. Exacerbations of the nasal and gastrointestinal disturbances occurred whenever he was in close proximity to a large quantity of the product, as when at the warehouse where the substance was stored, or whenever he was required to smell or taste the product in the course of his work. His personal and familial history was insignificant.

Routine scratch and intradermal tests, including those with the whole product and its various ingredients, gave strongly positive reactions only to milled psyllium seed and to the whole product. Nasal and ophthalmic tests were strongly positive only to these same atopens. A cytologic study of the nasal secretion taken following the nasal application of milled psyllium seed revealed numerous clumps of eosinophiles, as contrasted with a similar smear taken before the nasal test was performed. Skin tests to all other substances were uniformly negative. Clinically, the milled psyllium seed was more antigenic than the whole seed.

On one occasion following an intradermal injection with a saline extract of the psyllium seed, the patient developed a moderately severe reaction, manifesting both gastrointestinal and respiratory symptoms. These were easily controlled by two small subcutaneous injections of epinephrine, 1:1,000. Passive transfer tests were positive both to psyllium seed and to the whole product. Although the patient received large injections of a concentrated saline extract of psyllium seed,

which resulted in a large localized swelling at the site of injection, satisfactory hyposensitization was only temporary, and after a period of from three to four weeks, symptoms recurred on exposure to the offending allergen. Avoidance of contact tended to clear up the allergic symptoms within a few hours. Unfortunately, the patient's work required continued contact with the psyllium seed product, and further observation did not reveal improvement of his nasal or gastrointestinal atopy.

The above case induced us to do scratch testing with psyllium seed, in addition to the routine list of antigens in a series of 342 consecutive allergic persons. This series consisted of 212 patients with hay fever, twelve with vasomotor rhinitis, 109 with asthma, and nine with urticaria.

Positive reactions to psyllium seed were elicited in three allergic patients; one was troubled with vasomotor rhinitis, and the other two had hay fever. One, a 34-year-old female with hay fever, gave a clinical history of marked gastrointestinal sensitivity following the ingestion of either the whole or the milled psyllium seed. She stated that from fifteen to thirty minutes after its ingestion, marked cramps followed by a diarrhea would ensue. This had occurred on two separate occasions. She did not react to any other antigen except ragweed. The other hay fever patient also reacted very strongly to English plantain and showed marked clinical sensitivity to that pollen, but did not exhibit any clinical sensitization to psyllium seed.

DISCUSSION

The two patients manifesting clinical sensitivity to psyllium seed definitely stated that the ground form caused more symptoms than the whole seed. Inhalation of a small quantity of the milled psyllium seed produced marked sneezing and nasal turgescence in one of them. Ingestion of the whole seed failed to produce any nasal symptoms. Moreover, ingestion of the milled psyllium seed produced gastrointestinal disturbances sooner and with greater intensity than the whole seed.

Although only three in a series of 342 consecutive allergic persons gave positive reactions to psyllium seed, this substance should be considered an important allergen because of its extensive use. The laxative action of psyllium seed might obscure gastrointestinal symptoms which are allergic in nature, the unpleasant effects of which may be falsely attributed to the laxative action of the drug. In addition, if the drug should pass through the gastrointestinal tract in a very short period of time, all allergic symptoms may be temporary and slight.

SUMMARY

A case of psyllium sensitivity is presented.

Three persons in a series of 342 consecutive allergic persons gave positive reactions to psyllium seed. One of these manifested clinical sensitivity.

The milled psyllium seed is more antigenic than the whole seed.

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ALLERGIC SKIN REACTIONS TO MAMMALIAN SERA

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IN 1934, report was made of a case in which a general allergic reaction followed the intradermal injection of 0.1 c.c. of a 1:100 dilution of guinea pig serum in a patient also sensitive to horse serum.[1] Subsequent skin tests revealed that this patient was highly allergic to the sera of the horse, guinea pig, cow, sheep, hog, dog, rabbit, rat, mouse, cat, elephant, and opossum. His skin test was also positive to monkey serum but to a lesser degree.

Further study of the blood demonstrated the presence of skin-sensitizing antibodies (reagins) for each of these mammalian sera and showed also that a passively sensitized skin site, desensitized with guinea pig serum, no longer reacted to any of the other mammalian sera; a skin site desensitized with horse serum no longer reacted to cow serum and, vice versa, a site desensitized with cow serum did not react to horse serum. Circumstances made it necessary to discontinue these observations at this time, but it was decided to investigate these problems further in other serum-sensitive patients. Such patients were looked for in clinic and private practice during the past seven years.

In a group of approximately 3,630 patients having or suspected of having clinical manifestations of allergy and tested with the common inhalants, foods, and miscellaneous substances, including horse serum and horse dander, twenty-two were found who gave large skin tests (scratch method) to either horse serum, horse dander, or both. (In many other cases there were smaller reactions to these substances, but such cases were not included in this study.) A large skin test, in this case, means a wheal 10 mm. or more in diameter, the test having been made by the scratch method.

SKIN REACTIONS WITH VARIOUS BLOOD SERA

These twenty-two patients were tested by the scratch method with various animal sera diluted with an equal volume of glycerine. Included were fourteen mammalian sera* and two nonmammalian sera, namely, chicken serum and frog serum. These tests, as indicated in Table I, show that there was a great deal of variation in the reactions among different patients. For example, seven patients were sensitive to horse serum but not to any of the other sera with which they were

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*Porpoise serum was obtained through the courtesy of the Marineland Studios, Marineland, Florida; elephant serum was obtained through the courtesy of Franklin Park Zoo, Boston, Mass.

TABLE I

SKIN REACTIONS TO BLOOD SERA

(SCRATCH METHOD)

CASE NO.	DIAGNOSIS	HORSE DANDER	SERA												
			HORSE	COW	DOG	GUINEA PIG	HOG	RAT	RABBIT	MOUSE	SHEEP	CAT	FROG	CHICKEN	PORPOISE
1	Seasonal hay fever	–	++	+++	++++	++	++	++	++	+++	+++		–		
2	Seasonal hay fever	–	++++	–	–	–	–	–	–	–	–		–		
3	Seasonal hay fever	–	++	–	–	–	–	–	–	–	–		–		
4	Seasonal hay fever	+++	+++	–	–	–	–	–	–	–	–		–		
5	Seasonal hay fever	–	++++	–	–	–	–	–	–	–	–		–		
6	Seasonal hay fever	–	++++	–	–	–	–	+++	–	+++	+		–		
7	Asthma	++++	±	–	+++	–	–	–	–	++	–		–		
8	Asthma	++++	–	–	–	–	–	–	–	–	–		–		
9	Hay fever and asthma	±	++++	–	–	–	–	–	–	–	–		–		
10	Asthma	–	++++	–	+++	–	–	–	–	±	–		–		
11	Asthma	++++	–	–	–	–	–	+++	–	+++	–		–		
12	Hay fever, asthma and eczema	++++	++++	+++	++++	++++	++++	++++	++++	++++	++++	++++	++	–	
13	Perennial hay fever	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	–		
14	Asthma	–	++++	–	–	–	–	–	–						
15	Perennial hay fever and asthma	–	++++	–	++++	++	+++	++++	++++		–	++++	–	–	
16	Perennial hay fever and asthma	++++	++++	++++	++++	++++	++++	++++	++++			++++	–	–	++++
17	Perennial hay fever	–	++++	–	–	–	–	–	–					–	
18	Seasonal hay fever	++	++++	–	++++	++++	++++	++++	++++			++++	–	–	++++
19	Asthma	+++	++	+++	+++	++	++	++	+++		++++			–	
20	Asthma and hay fever	+	++++	++++	++++	++++	++++	++++	++++		++++			–	
21	Seasonal hay fever	–	+++	+++	++	+	+	–	++		++++			–	+++
22	Asthma	–	++++	++++	++++	++++	++++	++++	++++		++++			–	

Case 12. Tests were also positive to sera of elephant, opossum and to fish.

Case 13. Tests were also positive to sera of elephant, opossum and monkey.

±, Very small, questionable reaction; +, small reaction; ++, moderate reaction; +++, large reaction; ++++, very large reaction.

All gave negative tests to human serum.

tested; seven were sensitive to some degree to all the mammalian sera with which they were tested; three were sensitive to some degree to all the mammalian sera with which they were tested except one; and several were sensitive to two, three, or four sera but not to the others with which they were tested.

TABLE II

SKIN REACTIONS TO SERIAL DILUTIONS

SERA	CASE 15				
	1:10	1:100	1:1,000	1:10,000	1:100,000
Horse	++	+	–	–	–
Cow	–	–	–	–	–
Dog	++++	+++	++	–	–
Guinea pig	+	+	–	–	–
Hog	+++	++	±	–	–
Rat	+++	++	+	–	–
Rabbit	++++	+++	++	–	–
Sheep	+++	++	–	–	–
Chicken	–	–	–	–	–

SERA	CASE 16				
	1:10	1:100	1:1,000	1:10,000	1:100,000
Horse	++++	+++	+	–	–
Cow	++++	++	-	–	–
Dog	+++	+++	±	–	–
Guinea pig	++++	+++	–	–	–
Hog	++++	+++	–	–	–
Rat	+++	+++	±	–	–
Rabbit	+++	++	+	–	–
Sheep	++++	++	–	–	–
Chicken	–	–	–	–	–

SERA	CASE 18				
	1:10	1:100	1:1,000	1:10,000	1:100,000
Horse	++++	+++	++	±	–
Cow	–	–	–	–	–
Dog	++++	+++	+	±	–
Guinea pig	++++	+++	++	±	–
Hog	++++	+++	++	–	–
Rat	++++	+++	+	–	–
Rabbit	++++	+++	++	–	–
Sheep	++++	+++	+	–	–
Chicken	–	–	–	–	–

SERA	CASE 20				
	1:10	1:100	1:1,000	1:10,000	1:100,000
Horse	++++	++	+	–	–
Cow	+++	++	–	–	–
Dog	++++	+++	±	–	–
Guinea pig	++++	+++	±	–	–
Hog	+++	+	–	–	–
Rat	++++	+++	±	–	–
Rabbit	++++	++++	++	–	–
Sheep	++++	+++	+	–	–
Chicken	–	–	–	–	–

In several cases the results were not as anticipated and were not readily explained. For example, in two instances (Cases 15 and 18) tests were strongly positive to all mammalian sera with which tests were made except cow serum. In these cases all the tests were repeated at a later date with the same results. Furthermore, the same

preparation of cow serum, obtained from the same vial, later gave strongly positive tests on other patients (thus proving its potency). In several other cases, when the results appeared other than as anticipated, all tests were done two or three times with results which were practically identical at each repetition.

SKIN REACTIONS WITH SERIAL DILUTIONS

These patients were then tested with various mammalian sera in serial dilutions, in multiples of ten, from 1:10 to 1:100,000. The results indicated that, in some cases, there was considerable variation in the degree of hypersensitiveness to the various mammalian sera, as might have been anticipated from the varying size of the reactions to strong sera. In other cases, however, degrees of hypersensitiveness to the various sera were remarkably similar. Table II, illustrates these findings in four individual cases.

LOCAL PASSIVE TRANSFER*

Attempts to demonstrate the presence of reagins in the blood by local passive transfer were made in those cases in which tests were positive to many or all of the sera tested. The common method was employed, namely, the intradermal injection of 0.1 c.c. of the patient's serum into each of several skin sites of an apparently normal person. On the following day these sites were tested by intradermal injection of 0.02 c.c. of the test sera (1:10 dilution). The controls consisted of 0.02 c.c. of each test serum (1:10 dilution) injected into a normal skin site and 0.02 c.c. chicken serum injected into a previously prepared skin site. The results (Table III) indicate that positive skin tests were often, but not invariably, accompanied by the presence of transferable reagins. These results are in agreement with the findings in other cases of positive skin reactions in atopy in general. Passive transfer to cow and other sera was not prevented by the recipient's eating of beef between the time of injection of the reagin-bearing serum and the subsequent tests.

COMMENT

From a practical standpoint it is important to emphasize that a patient sensitive to one mammalian serum is likely to be sensitive also to other mammalian sera, and hence, in the administration of therapeutic or prophylactic serum, it is not safe to assume, for example, that a patient sensitive to horse serum will tolerate the serum of some other animal, such as the cow, rabbit, or sheep. The patient must be tested with *the serum to be administered.*

From a theoretical standpoint several interesting and important problems arise, especially the question as to whether or not some of

*New syringes were used in this work, a separate syringe being used for each specific test serum and never for any other.

TABLE III

SKIN REACTIONS ON PATIENTS COMPARED WITH REAGINS IN PATIENTS' BLOOD AS DETERMINED BY LOCAL PASSIVE TRANSFER TESTS

CASE NO.		SERA											
		HORSE	COW	GUINEA PIG	HOG	RAT	RABBIT	SHEEP	CAT	ELE-PHANT	DOG	FROG	CHICKEN
12	Skin reactions	+ + + +	+ + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	++	−
	Reagins	−	−	−					+ + + +				−
13	Skin reactions	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	−	
	Reagins	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +		−	
15	Skin reactions	+ + + +	−	+ +	+ + +	+ + + +	+ + + +	+ + + +	+ + + +		+ + + +		−
	Reagins	−	−	−	−	−	+ + +	−					−
16	Skin reactions	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +		+ + + +	−	−
	Reagins	+ + + +	+ +	+ + + +	+ + +	+ + +	+ + +	+ + +				−	−
18	Skin reactions	+ + + +	−	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +				−
	Reagins	+ + + +	−	+ + +	+ +	+ +	+ + +	+ +					−
19	Skin reactions	+ +	+ + +	+ +	+ +	+ +	+ + +	+ + + +			+ + +		−
	Reagins	+ + +	+	+	±	+ +	+ +	+ +			+ + +		−
20	Skin reactions	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +			+ + + +		−
	Reagins	+ +	+	+ + +	+ +	+ +	+ + +	+ + +					−
21	Skin reactions	+ + +	+ + +	+	+	−	+ +	+ + + +			+ +		−
	Reagins	+ +	+ + +	+	±	−	+	+ + + +					−
22	Skin reactions	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +			+ + + +		−
	Reagins	+ + +	±	+ + +	+ +	+ +	+ +	+ + +			+ + + +		−

these patients become sensitive to several or many sera by exposure to each individually, or whether, in some cases, exposure to one mammalian serum resulted in sensitivity to this particular serum plus several or many other mammalian sera due to the presence in such sera of one or more common allergens. It is intended to make this problem the subject of a further study and subsequent report.

SUMMARY

1. Among approximately 3,630 patients having or suspected of having clinical allergy there were twenty-two who gave large skin reactions to horse serum, horse dander, or both.

2. Skin tests with seven to fourteen different mammalian sera performed on these twenty-two patients demonstrated that (a) seven patients were sensitive to horse serum but not to any of the other sera with which they were tested, (b) seven were sensitive, to some degree, to all the mammalian sera with which they were tested, (c) three were sensitive to all the mammalian sera with which they were tested except one, and (d) several were sensitive to two, three, or four of the sera but not to the others with which they were tested.

3. Skin tests performed with serial dilutions of sera on patients who were sensitive to several or all the mammalian sera with which they were tested indicated that the degrees of hypersensitiveness to the various sera varied considerably in some cases while in other patients these degrees of hypersensitiveness were remarkably similar.

4. Local passive transfer tests indicated that positive skin reactions to the various sera were often, but not invariably, accompanied by transferable reagins.

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THE ABSORPTION OF INJECTED POLLEN ANTIGENS

A Comparison of the Subcutaneous and Intramuscular Routes

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SEVERAL years ago Feinberg and Bernstein[1] reported a method of measuring the rate of absorption of injected pollen extracts. Their method, briefly, was as follows: Persons nonsensitive to ragweed were injected intracutaneously with serial dilutions of a reagin-bearing serum taken from ragweed-sensitive patients. The following day the recipient was given an injection of ragweed pollen extract at a distant site, and the appearance time of the reactions at each of the previously sensitized sites was noted. Among a number of problems connected with antigen absorption which these investigators studied was a comparison of the intracutaneous and subcutaneous routes of injection. The experiment was repeated several times on each recipient, varying the route of the administration of the pollen extract, intracutaneous or subcutaneous, and keeping other factors constant.

Feinberg and Bernstein showed that intracutaneous injections were absorbed more rapidly than those given subcutaneously. Similar studies comparing the rate of absorption of pollen extract injected intramuscularly and subcutaneously were not made. In most standard works on immunity and allergy the statement is made that intramuscular injections are absorbed faster than subcutaneous ones. Thus Coca and his associates[2] stated, "Intravenous injection of an atopen is naturally the most dangerous as the entire dose reaches the shock organ at once. Intraspinous, intramuscular, subcutaneous, and intracutaneous methods rank in that order in the rapidity with which the injected substances are absorbed."

Most studies comparing the rate of diffusion of injected substances from subcutaneous and intramuscular sites have been carried out with horse serum-containing substances such as diphtheria antitoxin or pneumococcus serum. Working with these substances, all investigators are in agreement that absorption is faster from intramuscular than from subcutaneous sites. Many studies having to do with the rate of absorption of diphtheria antitoxin have been carried out by Park and his associates.[3-10] The problems investigated were: (1) the determina-

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Part of the expenses of this investigation was defrayed by a grant from the Children's Fund of Michigan.

tion of the antitoxin content of the blood after injection of diphtheria antitoxin, intramuscularly or subcutaneously, and (2) the ability of antitoxin injections given intramuscularly in some cases, and subcutaneously in others, to suppress the Schick test reaction.

Park and Williams[3] summarized their findings as follows: "It is naturally a matter of great importance as to how the antitoxin is given. When given subcutaneously the swelling caused by this injection rapidly disappears by the absorption of the water, but the globulins and antitoxin remain behind in the tissues because of the slow absorption of proteins. By testing many patients it has been found that it takes twenty-four hours for the major part of the antitoxin to be absorbed by the blood from the subcutaneous tissues and some twelve hours from the muscles. For total absorption it takes two or three days. Through the use of the Schick test it has been determined that an injection of antitoxin given intravenously passes out through the tissue fluids about ten times as rapidly as when the dose is given subcutaneously and four times as when given intramuscularly."

Subsequently Platou[11] determined the antibody content of the blood after giving antitoxin subcutaneously and compared his findings with Park and Williams' figures for the antitoxin content of blood after intramuscular and intravenous injections. He also found that antitoxin when given intramuscularly was absorbed faster than when given subcutaneously. Tuft[12] gave intramuscular and subcutaneous injections of horse serum to three persons and measured the horse serum content of the blood by means of precipitin tests. He found that the rates of absorption were practically the same.

Studies on the availability of specific pneumococcus antibody after intravenous, intramuscular, and subcutaneous injections were carried out by Tilghman and Finland.[13] They studied the circulating type-specific antibody and the development of skin reactions to type-specific polysaccharides and found that the highest circulating antibody titer was obtained and maintained when given intravenously. The maximum titers after intravenous injections appeared in five hours, after intramuscular injection in ten hours, and after subcutaneous injection in twenty-four hours. There were very wide individual variations among the subjects, and in a few cases the antibody titer seemed to be higher after the subcutaneous than after the intramuscular injections. There is no indication from their studies that this might have been due to variations in the protein content of the sera used.

Studies by Walbum[14] showed that the absorption of agglutinin from subcutaneous sites was much slower when the serum containing the agglutinin contained 15 per cent protein than when the serum used contained 6.2 per cent or less. When the more concentrated protein

solutions were diluted with normal serum, the rate of absorption from subcutaneous sites increased. However, when the protein content of the serum was markedly reduced, he found that the rate of absorption from subcutaneous tissues was retarded. A similar reduction in the protein content of the serum did not seem to retard the rate of absorption when the serum was injected into the muscles.

Studies on the rate of diffusion of injected antigen from various sites have been reported by Kahn.[15] He found that the rate of diffusion of horse serum injected into the muscle of a nonimmune animal was much faster than when the same amount was injected intracutaneously. He also found, in other experiments, that less diphtheria antitoxin was required to protect rabbits from toxin death when the antitoxin was injected intramuscularly than when it was injected subcutaneously. Kahn stated, "We are apparently not dealing with merely mechanical retention of the antitoxin by the tightly layered skin, since the antitoxin is similarly retained in the loose subcutaneous tissues. This similarity in the amounts of antitoxin retained by the intracutaneous and subcutaneous tissues, and the fact also that these loose subcutaneous tissues actually retain more antitoxin than skeletal muscle, for example, would indicate that the retention is not mechanical but biologic in character. We believe that the cutaneous tissue of an adult animal possesses the property of retaining or anchoring foreign substances, particularly proteins, as part of a non-specific mechanical mechanism of defense."

Several years ago, one of us[16] in a paper on the use of massive doses of pollen extract for the treatment of hay fever, suggested that the intramuscular injection of pollen extract was less likely to produce a general reaction than a similar dose given subcutaneously. This opinion followed the clinical observation of a large number of patients receiving pollen treatment. In view of the evidence previously cited, that intramuscular injections are absorbed faster than subcutaneous ones, it was felt that a reinvestigation of this problem, using aqueous pollen extracts instead of horse serum, would be advisable.

The method used was a modification of the one reported by Feinberg and Bernstein.[1] These investigators used the same patients for several observations. We used each recipient for only one experiment. We felt that there was a possibility that repeated pollen injections might produce an immune state in the recipient which would retard the rate of absorption in the latter experiments. Kahn[17] showed that there was a definite slowing of the rate of absorption in the immune animal in contradistinction to the nonimmune animal, affecting especially the rate of diffusion from the skin.

Blood serum was obtained from a patient who was sensitive to ragweed but not to any of the grass pollens. Serum of approximately the

same skin-sensitizing antibody titer was obtained from a patient sensitive to timothy pollen but not sensitive to ragweed. One-tenth cubic centimeter of each of these sera was injected intradermally into corresponding sites on the forearms of recipients* who were not sensitive by preliminary skin testing to either ragweed or timothy. Twenty-four hours later 2 c.c. of ragweed pollen extract was injected subcutaneously and simultaneously 2 c.c. of timothy pollen extract† was injected intramuscularly.‡

TABLE I

APPEARANCE TIME OF POSITIVE SKIN REACTIONS AT SITES PREVIOUSLY SENSITIZED WITH RAGWEED ANTIBODY AND TIMOTHY ANTIBODY

EXPERIMENT	FIRST OF PAIRED RECIPIENTS		SECOND OF PAIRED RECIPIENTS	
	MINUTES AFTER TIMOTHY EXTRACT GIVEN SUBCUTANEOUSLY	MINUTES AFTER RAGWEED EXTRACT GIVEN INTRAMUSCULARLY	MINUTES AFTER RAGWEED EXTRACT GIVEN ~~INTRAMUSCULARLY~~	MINUTES AFTER TIMOTHY EXTRACT GIVEN ~~SUBCUTANEOUSLY~~
1	5	10	4	12
2	6	8	9	15
3	10	11	9	15
4	7	11	4	12
5	12	17	8	21
6	13	14	12	20
7	x	x	x	x
8	14	17	11	18
9	10	10	9	12
10	14	19	x	x
11	12	14	11	17
12	7	19	x	x
13	9	21	13	15
14	16	17	16	22
15	14	16	x	x
Av. appearance time	10.6	14.5	9.6	16.2

x = no reaction probably due to inability to accept passive transfer.

At the same time another recipient similarly prepared as above was injected with the pollen extracts; the depth of the injections was reversed, the ragweed extract being given intramuscularly and the timothy extract, subcutaneously. These injections were given in sites approximately comparative. For example, the ragweed injection might be given in the right thigh 8 inches above the knee. The timothy injection in this recipient would then be given in the left thigh the same distance above the knee. After the injections of the pollen antigens, the appearance time of positive skin reactions at the previously sen-

*The recipients were children on the wards of the hospital between the ages of 8 and 12 years. These patients were convalescing from orthopedic procedures or had had pneumonia or other infections. All had been afebrile for several days and in most cases were ready for discharge from the hospital.

†The pollens were extracted in distilled water containing 5 per cent glucose and 0.5 per cent phenol and filtered through a Seitz filter after one week in the icebox. The protein nitrogen content was determined and the strength of the extracts so adjusted that the material injected contained 0.3 mg. protein nitrogen per cubic centimeter.

‡The intramuscular injections were made with a 22 gauge needle 1½ inches long. For the subcutaneous injections a 25 gauge needle ⅝ inch long was used.

sitized sites was noted. The experiments were done on paired recipients who were so selected that their age, weight, and height were approximately the same, in order to obviate other factors which might influence the rate of absorption. Fifteen pairs of recipients (thirty persons) were thus injected, and the appearance time of the positive skin reaction at the sensitized sites noted. Five recipients failed to give positive skin reactions in previously sensitized sites, indicating their inability to accept passive transfer of the skin-sensitizing antibody.

The results of these experiments are shown in Table I. The average appearance times of positive skin reactions in the sensitized sites were as follows: after ragweed extract given intramuscularly, 14.5 minutes; after ragweed extract given subcutaneously, 9.6 minutes; after timothy extract given intramuscularly, 16.2 minutes; and after timothy given subcutaneously, 10.6 minutes. There was an average of 4.9 minutes' retardation in the appearance time after the intramuscular injection of ragweed as compared to the subcutaneous injection of ragweed extract and an average of 5.6 minutes' retardation in the appearance time after the intramuscular injection of timothy extract as compared to the subcutaneous injection of timothy extract. In no case did a positive skin reaction develop at a sensitized site earlier after intramuscular injection than subcutaneous. In several cases only slight or no difference was noted. In other cases the difference was very marked, as much as 9 minutes in one case.

DISCUSSION

All previous observations comparing the rate of absorption of material injected into the muscles and into the skin have concerned themselves with horse serum or antitoxins for diphtheria or pneumonia prepared in horse serum. Those investigations have shown the rate of absorption from the muscle to be faster than from the skin. A possible explanation of our contradictory results is the fact that in these experiments we used an aqueous extract of pollen containing approximately 2 per cent protein (0.3 mg. protein nitrogen per cubic centimeter). Horse serum and antitoxins prepared in horse serum contain from 15 to 20 per cent protein.

As Walbum[14] has pointed out, the rate of absorption from the subcutaneous tissues is much slower when sera containing a high protein content are used. When the concentrated protein solution was diluted, the rate of absorption from the subcutaneous sites increased. The rate of absorption from the muscles was not affected by similar changes in the protein content of the serum. Kahn[15] has demonstrated that the skin possesses the property of retaining or anchoring foreign substances, particularly proteins. It is quite possible that the higher the

protein content of the injected material, the greater the anchoring effect of the skin. It is therefore conceivable that, when pollen extracts which contain relatively small amounts of protein are injected into the skin, the anchoring effect of the subcutaneous tissue does not come into play sufficiently to slow down the rate of absorption.

It is not necessarily true that a subcutaneous injection of pollen extract in a person under treatment for pollinosis would be absorbed more rapidly than an intramuscular injection. The possibility must be kept in mind that the reaction between the injected material and the sensitized tissue might result in the slowing down of the rate of absorption. That such is the case in the immune animal has been shown by Kahn's[17] work. It would seem that further study of this problem may be profitable.

SUMMARY

Evidence is presented that in the nonimmune, nonsensitive person aqueous pollen extracts are absorbed more rapidly after subcutaneous injection than after intramuscular injection.

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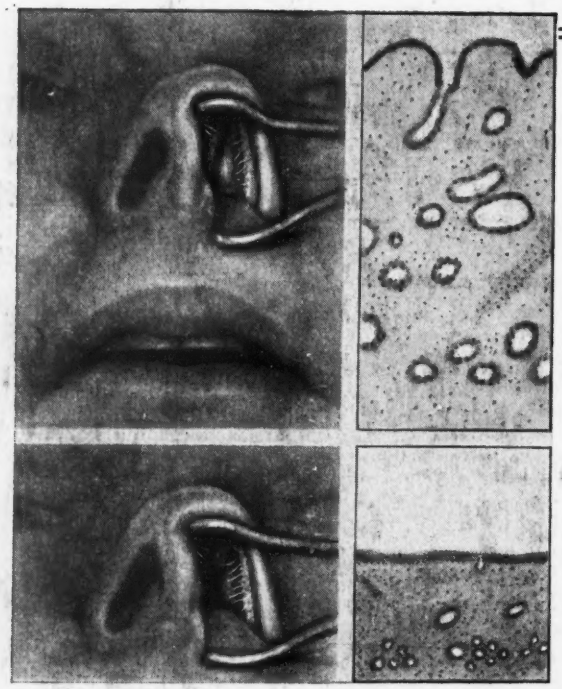

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